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Genetic Mapping of Quantitative Trait Loci Associated with Bioenergy Traits, and The Assessment of Genetic Variability in Sweet Sorghum (Sorghum bicolor (L.). Moench)

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GENETIC MAPPING OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH BIOENERGY TRAITS, AND THE ASSESSMENT OF GENETIC VARIABILITY IN SWEET SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

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Genetic mapping of quantitative trait loci associated with bioenergy traits, and the assessment of genetic variability in sweet sorghum (*Sorghum bicolor* (L.) Moench)

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Sweet sorghum, a botanical variety of sorghum is a potential source of bioenergy because high sugar levels accumulate in its stalks. The objectives of this study were to explore the global diversity of sweet sorghum germplasm, and map the genomic regions that are associated with bioenergy traits. In assessing diversity, 142 sweet sorghum accessions were evaluated with three marker types (SSR, SRAP, and morphological markers) to determine the degree of relatedness among the accessions. The traits measured (anthesis date [AD], plant height [PH], biomass yield [BY], and moisture content [MC]) were all significantly different ($P<0.05$) among accessions. Morphological marker clustered the accessions into five groups based on PH, MC and AD. The three traits accounted for 92.5% of the variation. There were four and five groups based on SRAP and SSR data respectively classifying accessions mainly on their origin or breeding history. The observed difference between SSR and SRAP based clusters could be attributed to the difference in marker type. SSRs amplify any region of the genome whereas SRAP amplify the open reading frames and promoter regions. Comparing the three marker-type clusters, the markers complimented each other in grouping accessions and would be valuable in assisting breeders to select appropriate lines for crossing. In

evaluating QTLs that are associated with bioenergy traits, 165 recombinant inbred lines (RILs) were planted at four environments in Nebraska. A genetic linkage map constructed spanned a length of 1541.3 cM, and generated 18 linkage groups that aligned to the 10 sorghum chromosomes. Fourteen QTLs (6 for brix, 3 for BY, 2 each for AD and MC, and 1 for PH) were mapped. QTLs for the traits that were significantly correlated, colocalized in two clusters on linkage group Sbi01b. Both parents contributed beneficial alleles for most of traits measured, supporting the transgressive segregation in this population. Additional work is needed on exploiting the usefulness of chromosome 1 in breeding sorghum for bioenergy.

DEDICATION

In Memory of My Uncle

Leepile John Botshake

This Dissertation is dedicated to my parents (Tlamme and Baitlhatswi Lekgari), my siblings, and the Lekgari family who continued to encourage and support me throughout my journey of seeking knowledge.

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CHAPTER 1

ASSESSMENT OF GENETIC VARIABILITY OF 142 GLOBAL SWEET SORGHUM ACCESSIONS USING MORPHOLOGICAL TRAITS AND MOLECULAR MARKERS

Assessment of Genetic Variability of 142 Global Sweet Sorghum Accessions using Morphological Traits, and Molecular Markers

Abstract

Sorghum bicolor (L.) Moench is the fifth most important crop in the world, and its agronomics and genetics have of recent drawn interest among scientists. Sweet sorghum, a variety of sorghum has the potential of becoming one of the sources of bioenergy because of the high sugar accumulation in its juicy stems. Exploring the diversity of sweet sorghum around the world is important in the development and improvement of the crop as a source of energy. In exploring the diversity of sweet sorghum, morphological and two types of molecular markers (simple sequence repeats [SSR] and sequence-related amplified polymorphisms [SRAP]) were used on 142 global sweet sorghum. The accessions showed a high significance ($P<0.05$) for all the morphological traits (anthesis date[AD], plant height [PH], moisture content [MC], and total biomass yield [BY]). The morphological markers clustered the accessions into five groups based mainly on PH, AD, and MC, with the principal component analysis (PCA) showing these traits to explain 92.5% of the total variation. The largest distant accessions were PI 571103 from Sudan and N99 from the United States. The Nei's genetic standard distances between accessions were calculated for the molecular marker data, and ranged from 0.024 to 1.135 and 0.078 to 0.866 for SSR and SRAP respectively. As expected, accessions of similar origin or breeding history had the lowest genetic distance (e.g. 'Mokula' and 'Marupantse' both from Botswana; NSL83777 and NSL83779 from Cameroon). Neighbor joining clustered accessions into five and four major groups using SSR and

SRAP respectively, and the clustering was based on their origin or breeding history. The clustering by all the three marker types showed some relationship in grouping the accessions, and seemed to compliment each other. The presence of accessions of different origin across clusters indicated similar genetics, and evidence of germplasm exchange between countries.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the crop species that can survive the harsh climatic conditions of the arid environments (Ritter et al., 2007). *Sorghum bicolor* sp *bicolor* contains both cultivated and wild races and possess a significant amount of genetic diversity for traits of agronomic importance (Hart et al., 2001). It is used as a source of grain food, syrup fuel, and feed for livestock. Sweet sorghum, a type of sorghum with high sucrose accumulation in their stems, has recently received attention as a source of biofuel (Ferraris, 1981). Sweet sorghums were selected to accumulate high levels of sucrose in the parenchyma of their juicy stems (Murray et al., 2009; Vietor and Miller, 1990). The stems are desired for food grade syrup, fresh chewing and alcohol production in Brazil (Murray et al., 2009), India and Africa (Vaidyanathan et al., 1987). In the US, sweet sorghum has been researched for biofuel for more than 30 years (Lipinsky, 1977), with primary research, development and breeding starting in the late 1970s (Murray et al., 2009) because of the high oil costs and need for alternative energy sources. Under favorable conditions, sweet sorghum crop is capable of producing up to 13.2 metric tones per hectare of total sugars, which is equivalent to 7682 liters of ethanol per hectare (Murray et. al., 2009). Sweet sorghum has a compelling advantage for cellulosic biofuel production over grain-based ethanol production, and its adaptation to marginal lands makes the per unit value of biomass production economical (Paterson, 2008).

Sweet sorghum gene pool creation had not received much attention mainly because it was not considered to be among important crops in the US, and the pedigree information is scarce and incomplete. Most sweet sorghums released in the US were

developed by public breeding programs in the 1900s and are mainly open pollinated cultivars (Swanson and Laude, 1934). The improvement was done mainly on syrup, sugar concentration and biomass, with lines primarily selected for improved disease resistance (Murray et al., 2009). Sweet sorghums were introduced to the US as landraces from Africa and China in the 1850s (Murray et al., 2009), and other cultivars were developed later, some with unknown origin. Genetic diversity or knowledge on patterns of diversity of genetic resources is of great importance (Warburton et al., 2001) and is a key component in crop improvement and plant breeding. The majority of the US released sweet sorghum cultivars have a narrow genetic base that can be traced to six African landraces (Murray et al., 2009). Currently there are no criteria (morphological traits or molecular markers) to differentiate sweet sorghums from grain sorghums (Murray et al., 2009), and most of the accessions lack the proper information to help distinguish between sweet and grain sorghum. Therefore when requesting sweet sorghum germplasm, one is limited to a few characters that are common in sweet sorghum like tall plants that are leafy (high biomass), and where available the brix degree, which also is subjective as there is no definite value for distinguishing grain sorghums from sweet ones. The Meridian, Mississippi Station tried curating what may be the world sweet sorghum collection, and when it closed, materials were transferred to the USDA sorghum collection in Griffin, GA (Freeman, 1979). Thus many diversity studies have concentrated on cultivars/lines that are common and known, leaving the vast majority of the collection (genetic sources) unexploited. In this study we tried to incorporate both the commonly used lines together with rarely used lines, and accessions from other sorghum collections.

The use of morphological traits in plants as markers for determining the genetic relationship dates back many years. Mendel followed visible phenotypic traits in progeny of sexual crosses, and the use of morphological markers has continued to the present day (Schulman, 2006). Phenotypic variables include continuous variables such as height, maturity, and yield as well as discrete variables like grain color, texture, insect and disease resistance (Franco et al., 2001). Franco et al. (2001) states that the truth underlying homogeneous groups or sub-populations of genotypes and their shape and structure is unknown because the association between the traits affects the shape of the groups and their structure is dependent on the composition of the group. However, clustering methods attempt to recover the true shape and structure of the sub-population. When using both the morphological and molecular marker data, two types of hierarchical classification are carried out independently. The morphological marker data first utilizes the computation of standard distances (e.g. Euclidean distances) and clustering strategies such as UPGMA or neighbor joining are applied, whereas with the molecular marker data, genetic similarities or dissimilarities using each band fragment as an attribute (0 for absence and 1 for presence) are determined then a clustering strategy applied (Franco et al., 2001). This enables genotypes to be clustered into groups that are as homogenous as possible. Phenotypic and genetic diversity are important in genetic conservation, evaluation and utilization of genetic resources, and the study of breeding germplasm for determining uniqueness and genetic constitution for the purpose of breeder's property rights (Franco et al., 2001; Ramakrishnan et al., 2004). The morphological markers are highly influenced by the environmental conditions, therefore there is a need to supplement or compliment their clustering with molecular marker data.

Polymerase chain reaction (PCR) is widely used in genomic DNA analysis, and one of its main applications has been in the development of DNA-based markers for map construction, breeding taxonomy, evolution and gene cloning (Li and Quiros, 2001; Schulman, 2006). Molecular markers are basically nucleotide sequences corresponding to a physical position in the genome, and their polymorphisms between accessions allow the pattern of inheritance to be easily traced (Schulman, 2006). The availability of molecular markers to assess diversity is a quicker way in helping breeders to select suitable lines/genotypes for crossing. The use of molecular markers as a tool to assess relatedness in and between cultivated and wild sorghum have been successfully used (Ahnert et al., 1996; Tao et al., 1993; Uptmoor et al., 2003; Menz et al., 2004; Anas and Yoshida, 2004; Ritter et al., 2007). PCR based markers are widely used in fingerprinting crops because of their high level of polymorphisms (Warburton et al., 2001), and their ease of detection (Sharon et al., 1997). Several PCR-based markers vary in their complexity, reliability and information generating capacity are available

Simple sequence repeats (SSR), also known as microsatellites, are based on tandem repeats of one to six core nucleotide elements. They are codominant markers dispersed throughout the genome, and have multiple alleles that often have conserved loci between related species (Brown et al., 1996; Schulman, 2006). Powell et al. (1996) stated that SSRs are able to discriminate among closely related individuals, and have advantage over other markers in their ability to trace pedigrees in plants. Therefore SSRs have been used in a variety of genetic studies like diversity analysis, quantitative trait locus mapping, gene tagging, and cultivar identification. In sorghum, several studies have been conducted involving either SSR markers alone or in combination with other marker

types (Casa et al., 2005; Perumal et al., 2007; Ali et al., 2008; Klein et al., 2008; Murray et al., 2009). Polymerase chain reaction made possible many other marker methods to be developed. Schulman (2006) indicated that there are those marker methods that detect specific, cloned and sequenced targets in the genome, while others use conserved or general primers that amplify from many anonymous sites throughout the genome.

Sequence-related amplified polymorphism (SRAP) markers are based on two primer amplifications that preferentially amplifies open reading frames (ORFs) or coding regions resulting in a number of dominant and codominant markers (Li and Quiros, 2001; Budak et al., 2004a; Ariss and Vandemark, 2007; Zhao et al., 2009). The forward primer amplifies the exon regions while reverse primer amplifies the intron and promoter regions (Li and Quiros, 2001; Zhao et al., 2009). Their polymorphisms result from the variation in length of these exons, introns, promoters and spacers both among individuals and species (Li and Quiros, 2001; Zhao et al., 2009). Sequence-related amplified polymorphism markers are more reproducible, stable and less complex (Han et al., 2008; Zhao et al., 2009) and more powerful in revealing the genetic diversity among closely related individuals than other marker types (Budak et al., 2004b), and have been used in a wide range of plant species like *Medicago sativa* (Ariss and Vandemark, 2007), *Brassica* (Li and Quiros, 2001), *Buchloe dactyloides* (Budak et al., 2004a,b), *Gossypium* (Lin et al., 2004), *Cucubita* (Ferriol et al., 2003), *Paeonia suffruticosa* (Han et al., 2008) and *Triticum spp* (Fufa et al., 2005; Zaefideh and Goliev, 2009). Ferriol et al. (2003) also reported that the information obtained from SRAP markers was in better agreement with the morphological variation and evolutionary history of morphotypes than that described by AFLPs.

Several diversity studies of sorghum and/or its wild relatives (Tao et al., 1993; Ahnert et al., 1996; Ali et al, 2008; Ritter et al, 2007) were limited to either grain sorghum or to germplasm from or within an individual country. In this era, germplasm exchange is an important factor in breeding as breeders try to develop modern cultivars with improved agronomic performance. The use of molecular markers has proven to be a good tool in assessing the genetic relatedness of different species (Ritter et al., 2007), and many types of markers have been used in sorghum. These studies have revealed both a wide and narrow genetic variation between agroecological zones. Folkertsma et al. (2005) indicated that there is a wide variability within accessions in the semi-arid Africa, but the south Asian accessions had a narrow diversity. Therefore, it is important to establish the genetic similarity among some of the world germplasm collection of sweet sorghum especially as its potential as an agro-industrial crop continues to draw more attention. Therefore the objectives of this study were to:

1. Examine the genetic variability within sweet sorghum accessions from different regions of the world for traits associated with bioenergy.
2. Classify/group the sweet sorghum germplasm based on SSRs, SRAPs and several morphological traits

Materials and methods

Plant material

One hundred and forty two (142) sweet sorghum accessions selected mainly based on brix reading were obtained from the USDA-ARS, University of Nebraska-Lincoln, NE; National Center for Genetic Resources Preservation (NCGRP), Fort Collins, CO; National Plant Germplasm System, Griffin, GA; Texas Agricultural System Station, College station, TX; University of Kentucky, KY; and the Department of Agricultural Research (Ministry of Agriculture), Botswana were used in this study (Table 1). These consisted of landraces, released improved cultivars and breeding lines. The pedigree information where available was obtained from GRIN website (http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl), ICRISAT website (http://www.icrisat.org), and other resource publications (e.g. Gorz et. al., 1990; Murray et. al., 2009), or accompanied the seed.

Agronomic traits

The 142 sorghum lines and two check cultivars (M81-E and Sugar drip) were planted under rainfed conditions at Mead during the 2009 season. The experimental design was an incomplete block design with 12 incomplete blocks of 12 entries each (12 x 12 alpha lattice) and two replications. Single row plots measuring five meters long with between row spacing of 0.75m were oversown at the rate of 160 000 seeds per hectare. The seeding rate was assumed to compensate for situation where there might be low viability of seed, and the final population density was on average 140 000 plant per hectare.

Four agronomic traits were measured and included anthesis date measured as the duration in days from planting to when 50% of the plants within a plot were shedding pollen; plant height measured as the distance from the base of the plant to the tip of the panicle; total biomass yield in Mg ha^{-1} when plants had reached their physiological maturity; and moisture content as the percentage difference between wet and dry biomass weight. Total biomass yield was calculated from a sample taken at harvest as follows: BY = (Dry weight of sample from 0.50m row)/(sample plot area in m^2) then calculated as Mg ha^{-1}. Plants were weighed immediately after cutting the 0.5m samples, bagged and placed into an oven at 120 – 160^oC for ten days to completely dry the samples. Samples were reweighed to obtain the dry weight.

DNA extraction and Marker analysis

Genomic DNA of each accession was extracted from fresh leaf tissues from plants planted in the greenhouse using cetyltrimethyl ammonium bromide (CTAB) protocol (Dweikat, 2005; Mahmood, 2004). The ground tissue was incubated in extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 1 M NaCl, 1% CTAB, 1mM 1,10-phenathroline and 0.15% 2-mercaptoethanol) at 65^oC for 1 hr; then equal volume of chloroform:isoamyl alcohol (24:1) added to the tissue mixture. After centrifugation at 3000 rpm, the supernatant was transferred to a new clean tube and DNA was precipitated with equal volume of cold isopropanol. DNA was air died at room temperature for an hour and then re-suspended in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) with 20ng RNase and incubated at 37^oC overnight. Equal volume of 24:1 Chloroform:isoamyl alcohol was added to the DNA-RNase mix and centrifuged at 3000 rpm for 5 minutes, the resulting

supernatant was transferred to new tube. Two volumes of cold absolute ethanol and 5µl of 8M ammonium acetate were added to the supernatant in order to precipitate the DNA. After centrifugation, DNA pellets were air dried at room temperature, and later re-suspended with 200 - 400 µl TE buffer depending on the size of the pellet. DNA concentration was determined using a spectrophotometer (TKO 100 Fluorometer, Hoefer Scientific Instruments, San Francisco, CA).

A collection of 82 oligonucleotide primer pairs that included 33 sorghum SSRs (Schloss et al. 2002; Lubbock, TX unpublished) and 49 SRAP combinations (Li and Quiros, 2001; Riaz et al., 2001) were synthesized, and marker assays were conducted following the procedure of Kuleung et al. (2004). A 25 µl total/reaction was used, consisting of 75 ng genomic DNA, 100 ng primer pair, 125 µM dNTP, 50 mM KCl and 10 mM Tris-HCl, 2mM $MgCl_2$, and 1 unit Taq polymerase. The amplification procedure consisted of one cycle at 94°C for 3 min, followed by 35 cycles of 1 min at 94°C, 1 min at 55 to 58^{o}C for SSRs depending on the primer pair, and 47^{o}C for SRAPs, 1 min at 72^{o}C, and final extension step at 72°C for 5 min. The reaction was then cooled to a resting temperature of 4^{o}C and resolved by electrophoresis in a 12% non-denatured polyacrylamide gels (37:1 acrylamide:bis-acrylamide). The gels were stained in 1 µg/ml ethidium bromide for 10 min, distained in deionized water for 15 min, then photographed using the Gel Doc2000 (Bio-Rad, Hercules, CA.).

Data Analysis

Analysis of variance was performed on agronomic data using PROC MIXED where incomplete blocks were treated as random effects, and then principal component analysis using a correlation matrix based on least square genotypic means (LSMEAN) was done using PROC PRINCOMP to determine the traits that account for most variation between lines. Pearson correlations were done on the least square genotypic means for the four agronomic traits. Because of the differences in the units of the traits, agronomic data were standardized using the standard deviation of mean by PROC STANDARD and then used for clustering by PLOC CLUSTER with average linkage based on Euclidean distance of the standardized variables (Flury and Riedwyl, 1986). Dendrograms were constructed using PROC TREE (SAS, 2008).

The Nei's (1972) genetic distance and neighbor joining clustering methods were used for the molecular marker data to determine how the sorghum accessions grouped using a band scoring of "1" to indicate the presence of an allele and "0" when absent. Polymorphism information content (PIC) values were calculated as in Anderson et al. (1993) who assumed homologous alleles. Polymorphic information content for a locus is calculated as:

$$PIC = 1- \Sigma P_{ij}^{2},$$

where P_{ij} is the relative frequency of the j^{th} allele of the i^{th} locus, summed over all the alleles for individual marker locus over all lines. A marker with a PIC value of more than 0.5 is considered to be highly informative, between 0.25 and 0.5 as informative and less than 0.25 as slightly informative (Botstein et al., 1980). The genetic diversity was estimated by similarity index calculation from band sharing data of each pair of DNA

fingerprints. The molecular markers data analysis was achieved through a series of diversity study software. Population software v.1.2.30 (http://bioinformatics.org/~tryphon/populations), was used to generate similarity matrix used to construct a similarity dendrogram by cluster analysis using Neighbor Joining method to determine how sorghum accessions were related. The Population software generated the genetic distances based on Nei (1972) standard genetic distance:

$$Ds = -ln(Jxy/\sqrt{JxJy})$$

where $Jx = \Sigma\Sigma\ Xij^2/r$, $Jy = \Sigma\Sigma\ Yij^2/r$, and $Jxy = \Sigma\Sigma\ X_{ij}Y_{ij}/r$ with X_{ij} and Y_{ij} being the frequencies of allele *i* at *j* locus of populations X and Y, respectively (Takezaki and Nei, 1996). Population software was also used for dendrogram construction and viewed using the TreeView (Win32) software version 1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod /treeview.html; Page, 1996).

Results and Discussion

Agronomic traits

Harvest was accomplished approximately at the same time for all lines, and due to the wide range of maturity that exist among the lines, some were past the physiological maturity stage at the time of harvest. The analysis of variance showed highly significant differences ($P<0.01$) for all the traits measured among sorghum accessions (Table 2; Appendix 1). The wide variation in traits measured exhibited by accessions suggest that the diversity exist among sorghum in the world. The anthesis date showed a wide range of maturity (70 to 147 days to anthesis) (Table 4), plant height ranged from 76.0 cm to 423.8 cm, moisture content ranged from 45.4 to 80.6%, and total biomass yield (dry

matter) ranged from 3.81 $Mgha^{-1}$ to 59.19 $Mgha^{-1}$ (Table 4). PI 276804 which had the lowest total biomass is an Ethiopian landrace with moderate tillering and medium height (ICRISAT website), while N99 an F_7 selection from a cross between a Fremont forage sorghum and Theis sweet sorghum (Gorz et. al., 1990), produced the, highest biomass yield . Theis is a high biomass producer that may have contributed to N99's high biomass yield. There were highly significant correlations between anthesis date and plant height (r=0.53***), anthesis date and total biomass yield (r=0.57***), and plant height with moisture content and plant height with total biomass yield (r= 0.285*** and 0.712*** respectively) (Table 3). These correlations were somehow expected because of the traits involved, studies have shown that late maturing crops tend to be taller than early maturing ones. Late flowering/maturity plants that are tall will also accumulate more of the photosynthates leading to their higher biomass accumulation compared to their shorter and early counterpart. The highly significant correlations also suggest that it will be difficult to select for or against one trait without impacting the other. Cultivars of variable maturity are desired when selecting for taller and high biomass yielding plants for bioenergy in order to minimize storage cost associated with high volume of wet biomass (high moisture vegetative matter from sweet sorghum). The results also suggest that high biomass yielding accessions contain high amount of juice (moisture content), that is desirable for immediate fermentation for bioethanol production.

Cluster analysis based on similarities and rooted tree diagram grouped the lines into five main clusters (Fig 1; Appendix 3 - 7). Although the agronomic traits did not distinctly group lines according to their geographic origin/area, materials from the same area tended to cluster together within each group indicating that their origin played a role

in the selection or development of germplasm used (Fig 1; Appendix 3 - 7). Apart from the germplasm origin, the lines grouped together mainly according to plant height, anthesis date and percent moisture content. For example, group 1 consisted of materials that were on average 248.0 cm tall (176.3 – 288.3 cm) (Appendix 3); group 2 averaged 328.5 cm (287.3 – 390.5 cm) (Appendix 4); group 3 averaged 399.5 cm (379.2 – 423.8 cm) (Appendix 5); group 4 averaged 170.7 cm (104.3 – 200.8 cm) (Appendix 6); and group 5 was on average 123.8 cm tall (76.0 – 281.8 cm) (Appendix 7). The above reasoning was also supported by data obtained from the principal component analysis in which anthesis date, plant height, biomass and moisture loss accounted for the 92.5% of the variation (i.e. principal components 1, 2 and 3) (Table 5). The two furthest distance exist between accession PI 571103 (a landrace from Sudan) and N99 with distance of 7.818, while the closest accessions were PI 569520 (a breeding line from Sudan) and ICSR90017 (a restorer line from ICRISAT) with a distance coefficient of 0.189. The clustering based on these traits shed some lights on what traits were important during selection of these sweet sorghum accessions. Apart from the early breeding strategies for tall and bushy lines as suitable sweet sorghum, the height may also reflect the effect the environment had on the accessions from different agroecological zones (e.g. due to photoperiod sensitivity).

Molecular marker data

Based on the 33 SSR marker pairs screened, 29 produced 84 polymorphic alleles with a mean value of 2.90 alleles per marker locus (Appendix 8). This was lower than the 3.22 observed by Ali et al. (2008) or 3.4 observed by Schloss et al. (2002). The

polymorphic information content of SSR markers ranged from 0.22 to 0.75 with mean value of 0.52 (Table 6). These values were higher than those of 0.40 and 0.44 observed by Ali et al. (2008) and Folkertsma et al. (2005), respectively. The differences may be attributed to the number of bands/alleles scored and the type of SSR markers used. For example, Ali et al. (2008) used only one SSR marker type (*xcup*) whereas in this study different SSR marker types were used, and the number of alleles/bands scored per marker differs between individual studies. The mean PIC value of 0.52 indicates that the markers used were highly informative according to Botstein et al. (1980) who suggested that markers with PIC >0.5 are considered highly informative. Ali et al. (2008) using 72 US sorghums, reported a PIC value range of 0.03 to 0.87.

For each pairwise similarity estimate (i.e. similarity between two accessions based on molecular marker data), a dendrogram was constructed using the Nei's standard genetic distance (1972). The accessions were grouped mainly according to their origin or breeding history (Figure 2; Appendix 9). The Nei's standard genetic distance ranged from 0.024 to 1.135, with 'Marupantse' and 'Mokula' having the smallest genetic distance (0.024) while PI 154844 and NSL 55404 exhibited the largest genetic distance (1.135) followed by NSL 55429 and NSL 87920, and PI 602982 and PI 571103 with a value of 1.099. 'Marupantse' and 'Mokula' are both from Botswana, and 'Marupantse' is an advanced/improved cultivar while 'Mokula' is of unknown parentage, but the two do not belong to the same sorghum race (kafir vs durra-caudatum) suggesting a close relationship among sorghum races. PI 154844 is a landrace from Uganda while NSL 55404 is from India. These two however belong to the Durra race. NSL 53429 is a landrace from India, while NSL 87920 is from Cameroon; PI 602982 is a line developed

in Mali with pedigree (SPV 35/E35-1)/CS 3541), and PI 571103 is a landrace from Sudan.

Cluster analysis grouped the accessions into five major groups using Nei's standard genetic distances (1972) through neighbor joining (Fig 2; Appendix 9). Group 1 consisted of germplasm mainly from East Africa (Sudan, Kenya, and Ethiopia), while group 2 consisted of germplasm from different regions. Group 3 consisted of Nebraska lines (released and breeding lines) and some Ethiopian germplasm. The largest group was group 4 which consists of 47 accessions, and occupied mainly by germplasm from Botswana and the US (especially Nebraska) (Appendix 9). The SC accessions from Botswana might have been part of the 1960s USDA sorghum conversion (SC) program, thus creating a link between Botswana sorghums and the US sweet sorghums, e.g. is 65D which is an introduction to Botswana from USA with unknown parentage. The last group was the smallest (9 accessions) and contains germplasm from different regions. Within each major group, accessions from similar/same country/region grouped together to form smaller clusters. This is in agreement with Wang et al. (2009) and Murray et al. (2009) who observed that both sweet and grain sorghums germplasm corresponded well with the geographic locations where the accessions originated. Since most of the accessions used were landraces with unknown parentage, it can only be assumed that accessions with same origin may be highly related. However, those with known parentage like the Nebraska breeding lines, the ones with similar pedigree tend to cluster together. For example, the lines that have "wheatland" in their parentage (05C09882(5) tan, 05C09881(4) ppbmr, and 05C09892(6) ppbmrsw, etc) were closer to wheatland, while lines like 05C09889(1) vtallsw grouped with N99. Ali et al. (2008) reported that Dale,

N108, Theis, Cowley and Norkan clustered in the same major group but different subgroups, in this study they were also clustered in the same major group (Appendix 9). The within cluster subgroups of materials from the same region further shows the individual selection of each breeding program's in sweet sorghum improvement.

Out of the 49 SRAP marker pairs screened, 40 polymorphic pairs produced 109 alleles, with a mean of 2.73 alleles (Appendix 8). This value was lower than that of SSR markers, however, the PIC for SRAP was higher than that of the SSR because SRAP markers had lower allele frequency that ranged from 0.15 to 0.94 with mean of 0.56 (Table 6). The pairwise similarity estimate using Nei's standard genetic distance ranged from 0.078 (NSL 83777 and NSL 83779) to 0.866 (PI 286245 and Orange). Both NSL 83777 and NSL 83779 are sorghum landraces from Cameroon, while PI 286245 is from Sudan and 'Orange' had no clear origin as there are various versions of 'Orange' with different origins. The SRAP markers also grouped the accessions according to their origin or breeding history although the groups were different from the SSR groups. The differences between markers in clustering the accessions may be due to differences in genomic regions amplified by each marker type.

Cluster analysis based on neighbor joining using Nei's standard distances produced four major groups (Figure 3; Appendix 11). Group 1 consisted of accessions from East and West Africa and had 59 accessions. Group 2 was the smallest with 9 accessions mainly from East Africa. Group 3 consisted of germplasm from both Botswana and the Americas. Unlike the SSRs grouping, the Nebraska lines that featured in this group were very few and were mainly the released ones. The final group (group 4) consists mainly of the Nebraska breeding lines with some ICRISAT and India accessions.

The SRAP markers seem to have separated the accessions well based on their breeding and origin compared to SSRs. Budak et al. (2004b) reported that SRAP markers are good in showing true variation within and among buffalograss cultivars. Zhao et al. (2009) also observed SRAPs clustering seemed to agree with morphological classification, although that was not the case with this study. This study may have been limited by the number of morphological traits measured as well as the number of field experiments conducted. Ritter et al. (2007) when looking at other types of molecular markers observed that clusters developed based on agronomic data could not approximate groupings produced by molecular markers. When looking at within group clustering, one could observe smaller subgroups aligned to each country or breeding program (Appendix 11). The differences between the marker clusters could be due to the differences in marker type. Simple sequence repeats amplify randomly in the genome whereas the SRAP amplify from the open reading frames or promoters of the genes. Therefore, SRAPs may be more informative in grouping accessions based on their breeding history.

Looking at the clusters for the three data types, it has been shown that each data type has its own strength but all seem to reflect the breeding history of the germplasm. The morphological data grouped accessions mainly based on plant height which is one of the characters that breeders base their selection on. Therefore, what the molecular markers (esp. SRAP) showed was what potential genes were selected for in these accessions. Wang et al. (2009) reported that most of agronomic traits are affected by different levels of population structure; therefore may contribute to the differences observed between clustering conducted with different marker data. Perumal et al. (2007) suggested that a more comprehensive and composite index based on pedigree,

morphological, biochemical and molecular data is expected to improve accuracy of grouping individuals. Selection based on one morphological trait can impact several other traits (especially those traits that are highly correlated) because there may be one gene involved in regulating different traits of importance (epistasis). Therefore, the differences and similarities in clustering by different marker types could also be attributed to differences in selection pressure during the development or selection of each accession.

Conclusion

The agronomic and molecular marker data produced distinct cluster groups for the sorghum accessions evaluated. The groups are not the same per se, but seem to compliment each other because accessions from the one region tend to cluster together in one major group as one move from the morphological data to the SRAPs data. For example, Botswana accessions appeared in three major groups (1, 2 & 3) based on morphological data; were in one major group (4) but four different subgroups based on SSRs; and were grouped in one major group (3) with one main subgroup based on SRAPs data. The above results compliment each other because together they can help breeders in choosing what accessions to use in crosses for sweet sorghum improvement. The molecular marker data will help select genetically distant accessions for crossing, while morphological data will enable breeders to plan for a successful crossing (e.g. similar height, anthesis dates, etc). The agronomic data clusters provide clues to which characters were important is separating individual accessions, while SSRs clusters further narrowed the groups based on their origin. The SRAP markers then even refined the

groups as they showed the breeding pattern/history of the accessions. This study also showed that there was a considerable amount of germplasm movement across different regions of the world, and there is still a large genetic diversity even within some regions. For example, some of the lines that were genetically distant from each other came from the same region. Previous reports have also indicated that the diversity of sorghum is limited in certain regions compared to others. Therefore, sorghum improvement will benefit from this wide range of diversity, and germplasm exchange will be the key to the success of improving sweet sorghum cultivars as a source for biofuel. This study has also strengthened the point that the use of molecular markers to compliment the agronomic data where pedigree information is limited or unavailable is essential and beneficial to plant breeders.

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Table 1. List of germplasm accessions used in diversity study, their origin, year of registration and parentage.

Entry	Name	Designation/accession no.	Registration Year	Place of origin	Parentage/Pedigree
1		PI 217892	1954	Sudan	
2		PI 246698	1958	India	
3		PI 276804	1961	Ethiopia	
4		PI 286245	1963	India	Sudan collection
5		PI 287611	1963	Zimbabwe	
6		PI 329336	1968	Ethiopia	
7	Durra	PI 329761	1968	Ethiopia	
8		PI 562943	1992	Sudan	Landrace
9		PI 569009	1993	Sudan	Wild collection
10		PI 569154	1993	Sudan	Landrace
11		PI 569283	1993	Sudan	Landrace
12		PI 569295	1993	Sudan	Landrace
13		PI 569520	1993	Sudan	Cross 45/6
14	PN 4135	PI 569590	1993	Sudan	PN 4135 (Breeding line)
15	PN 4288	PI 569597	1993	Sudan	PN 4288 (Breeding line)
16	PN 5043	PI 569644	1993	Sudan	PN 5043 (Breeding line)
17	PN 6058	PI 569670	1993	Sudan	PN 6058 (Breeding line)
18	Waramsara	PI 570717	1993	Sudan	Landrace
19	Mesera	PI 570718	1993	Sudan	Landrace
20	Sinidyl	PI 570731	1993	Sudan	Landrace
21	Thok brown	PI 570747	1993	Sudan	Landrace
22	Ani-el-gaong	PI 570753	1993	Sudan	Landrace
23	SBI 100	PI 570759	1993	Sudan	
24	UT 69	PI 570761	1993	Sudan	
25	Maluk	PI 570775	1993	Sudan	Landrace
26	Wad akar 9	PI 570877	1993	Sudan	Landrace
27	Feterita	PI 570957	1993	Sudan	Landrace
28	Kawanda L53	PI 571067	1993	Sudan	Landrace
29	Kawanda L31	PI 571068	1993	Sudan	Landrace
30	Msambiji	PI 571073	1993	Sudan	Landrace
31	Zerazera	PI 571120	1993	Sudan	Landrace
32	Kalili	PI 571126	1993	Sudan	Landrace
33	Karinaka	PI 571176	1993	Sudan	Landrace
34	A 154	PI 571276	1993	Sudan	Landrace
35	A 211	PI 571284	1993	Sudan	Landrace
36		PI 571370	1993	Sudan	Landrace
37	Wad bashir 3	PI 586791	1967	Sudan	Landrace
38		NSL 50393 (PI 651101)	1968	Indiana	Landrace
39		NSL 54316	1967	Uganda	Breeding line
40		NSL 55404	1967	India	Landrace
41		NSL 55429	1967	India	
42		NSL 55431	1967	India	

Table 1 cont'd

43	EC 21415	NSL 55645	1967	Uganda	
44	Hundi jowar	NSL 76942	1970	India	Landrace
45		NSL 82099	1972	Cameroon	Landrace
46		NSL 83601	1973	Cameroon	Landrace
47		NSL 83611	1973	Cameroon	Landrace
48		NSL 83656	1973	Cameroon	Landrace
49		NSL 83777	1973	Cameroon	Landrace
50		NSL 83779	1973	Cameroon	Landrace
51		NSL 83984	1973	Cameroon	Breeding line
52		NSL 87920	1974	Cameroon	Landrace
53		NSL 92446	1976	Ethiopia	Landrace
54		NSL 92465 (Orange-red)	1976	Ethiopia	Landrace
55		NSL 103374	1979	Cameroon	Landrace
56		NSL 92465 (White)	1976	Ethiopia	Landrace
57		NSL 92465 (Red)	1976	Ethiopia	Landrace
58	Green leaf	NSL 4028	1955	Texas	Leoti-Sudan 2/Leoti-Sudan 4
59	Roma		1993	South Africa	Sudan grass type variety grown in Texas
60	Theis	CSR 216	1978	Mississippi	(Wiley/C.P. Special)/(MN1054/White African)/MN660
61	Dale	NSL 74333	1973	Mississippi	Tracy/MN960 (PI 152857)
62	Cowley	NSL 189405	1985	Texas	Mer.64-7/Mer.64-6 (F2 selection)
63	05CO9810 (4) F3		2005 - nusery	Nebraska	
64	Mall			Botswana	Sweet sorghum collection
65	SC - 154			Botswana	Sweet sorghum collection
66	PMC - 18	PI 510906	1980	Botswana	Landrace
67	PMC - 5	PI 510893	1980	Botswana	Landrace
68	SC - 163			Botswana	
69	SC - 15			Botswana	
70	SC - 161			Botswana	
71	SC - 157			Botswana	
72	PSA - 160	PI 511004	1980	Botswana	Landrace
73	PMK - 80	PI 510942	1980	Botswana	Landrace
74	IPWA 1	IS 19674	1975	Zimbabwe	Landrace
75	A 157	IS 9890	1974	Sudan	Landrace
76		IS 22636	1980	Cameroon	Landrace
77	Ikumba	IS 20962	1979	Kenya	Landrace
78	Evsitu (short)	IS 21005	1979	Kenya	Landrace
79		IS 21991	1979	India	Landrace
80	Andiwo ma rabour	IS 21229	1979	Kenya	Landrace
81	Ochuti ma rabour	IS 21235	1979	Kenya	Landrace

Table 1. cont'd

82	Sabina	IS 20984	1979	Kenya	Landrace
83	Andiwo	IS 21100	1979	Kenya	Landrace
84	Andiwo ma rabour	IS 21260	1979	Kenya	Landrace
85	Hegari 6645-27-1-4-2	IS 131	1974	Ohio, USA	Hegari 6645-27-1-4-2
86		IS 20888	1979	Angola	Breeding line
87	Olusi	IS 20963	1979	Kenya	Landrace
88	Sabina	IS 20974	1979	Kenya	Landrace
89	N98 short	PI 535783	1990	Nebraska	(Waconia//AN39/N4692-Rio)/Fremont
90	N98 tall	PI 535783	1990	Nebraska	(Waconia//AN39/N4692-Rio)/Fremont
91	N99	PI 535784	1990	Nebraska	Fremont/Theis
92	N100	PI 535785	1990	Nebraska	Waconia/Wray
93	N108	PI 535793	1990	Nebraska	Inbred derived from Saccharum sorgo
94	Wheatland	CIso 918	1936	Oklahoma	Milo/Kafir
95	Norkan	NSL 4002	1942	Kansas	Atlas/Early Sumac
97	ICSR56	IS 84, IS 517		ICRISAT	Restorer line
98	ICSR160	IS 84, IS 517		ICRISAT	Restorer line
99	ICSR196	IS 84, IS 517		ICRISAT	Restorer line
100	ICSR90017	IS 1055		ICRISAT	F1 MS/Jowar BP53(MS/IS 1055) - Restorer line
101	ICSRP3034			ICRISAT	Restorer line
102	ICSV700	IS 3443		ICRISAT	Restorer line
103	S35	PI 602982	1980	Nigeria	(SPV 35/E35-1)/CS 3541
104	E36-1			Ethiopia	
105	NTJ2				
106	Seredo			Kenya	
108	Grassl	PI 154844 01 SD	1946	Uganda	Introduced as 'Lwera'
109		PI 175919 01 SD	1949	Maryland	Turkey
110	Suki	PI 217768 02 SD	1954	Sudan	
111	Chinese Amber	PI 22913 04 SD	1908	China	
112	Chinese Amber	PI 248298 01 SD	1958	India	
113	Mf.G.F.:383	PI 257294 02 SD	1959	Argentina	
114	Mf.G.F.:581	PI 257295 03 SD	1959	Argentina	
116	Perennial sweet Sudan	PI 562717 01 SD	1992	Texas	
118	Ajax Sweet	PI 571103 01 SD	1993	Sudan	
119		PI 591038 01 SD	1995	Nigeria	
120		05C09880-1(2)	2006 - nursery	Nebraska	(ms7//Tx430)/mix of Dale, Wray & Sugar Drip
121		05C09881msTAN	2006 - nursery	Nebraska	(128ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip

Table 1. Cont'd

122		05C09889-1-3tall tan	2006 - nursery	Nebraska	(122ms3/Wheatland-bmr12)/Dale
123		05C09890(1) PP bmr	2006 - nursery	Nebraska	(122ms3/Wheatland-bmr12)/Dale
124		05C09892 (3) - 2 tanmedbmr	2006 - nursery	Nebraska	(128ms3/Wheatland-bmr6)/Wray
125		05C09654(3) sw	2006 - nursery	Nebraska	(ms3/Wheatland-bmr6)/C297
126	65D			Botswana	Unknown introduction from USA
127	Kanye standard	PI 540519		Botswana	Landrace
128	Marupantse	PI 540516		Botswana	Landrace
129	Mokula			Botswana	Landrace
130	Segaolane	PI 540518		Botswana	Landrace
131	Sureno	PI 561472		Hunduras	[(SC423/CS3541)E35-1]-2
132		05C09882(1)tanbmr	2005 - nusery	Nebraska	(128ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
133		05C09882(3) tanbmr	2005 - nusery	Nebraska	(128ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
134		05C09889(1) vtallsw	2005 - nusery	Nebraska	(122ms3/Wheatland-bmr12)/N99
135		05C09892(3)-4 tallbmr	2005 - nusery	Nebraska	(122ms3/Wheatland-bmr12)/N99
136		05C09880(3)tan	2005 - nusery	Nebraska	(ms7//Tx430)/mix of Dale, Wray & Sugar Drip
137		05C09881(4)ppbmr	2005 - nusery	Nebraska	(128ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
138		05C09882(5) tan	2005 - nusery	Nebraska	(128ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
139		05C09882(8) tanbmr	2005 - nusery	Nebraska	(128ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
140		05C09882(9) tanOP	2005 - nusery	Nebraska	(128ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
141		05C09892(6) ppbmrsw	2005 - nusery	Nebraska	(128ms3/Wheatland-bmr6)/Wray
142		05C09892(3) tanbmr	2005 - nusery	Nebraska	(128ms3/Wheatland-bmr6)/Wray
143		05C09891(2) bmr	2005 - nusery	Nebraska	(122ms3/Wheatland-bmr12)/N98
144	SN372 - Chinese Amber			Texas	
145	Orange				
146	Blackstrap			Kansas	

Table 2. Mean squares of anthesis date (AD), plant height (PH), moisture content (MC) and total biomass (BY) measured at Mead in 2009 season

Source	DF†	AD	PH	MC	BY
Rep	1	4.53	1961.99	122.45***	9.87
Block(Rep)	22	9.82	1171.73**	19.95**	99.98**
Line	138	282.67***	9346.66***	66.21***	186.29***
Residual	112	7.18	665.85	9.54	47.92
Mean		**92.4**	**273.9**	**64.3**	**24.06**
CV (0.05)		**2.91**	**9.42**	**4.83**	**28.77**
Range		**69.5-147.2**	**76.0-423.8**	**45.4-80.6**	**3.81-59.19**

† - The degree of freedom for Lines was less than expected because of some missing data

, * indicate significance at probability values of 0.05 and 0.01 respectively

Table 3. Correlation coefficients of anthesis date (AD), plant height (PH), moisture content (MC) and total biomass (BY) measured at Mead in 2009 season (n=142).

	AD	PH	MC	BY
AD	1			
PH	0.530***	1		
ML	0.054ns	0.285**	1	
DM	0.570***	0.712***	-0.005ns	1

*** indicates significance at probability value of 0.01 respectively

ns - indicates non significance at P<0.05

Table 4. Least square means of anthesis date (AD), plant height (PH), moisture content (MC) and total biomass (BY)for the 142 accessions evaluated at Mead in 2009.

Accession number/name	AD (days)	PH (cm)	MC (%)	BY ($Mgha^{-1}$)
PI 217892	81.9	278.3	67.8	18.21
PI 246698	92.5	288.3	66.8	29.23
PI 276804	76.7	161.2	67.4	3.81
PI 286245	98.5	262.7	60.1	22.67
PI 287611	116.6	380.1	60.2	41.86
PI 329336	121.5	338.0	67.5	38.51
PI 329761	101.3	247.2	67.6	24.56
PI 562943	96.8	336.1	57.4	49.32
PI 569009	93.8	356.2	57.0	52.40
PI 569154	85.7	248.5	66.3	8.59
PI 569283	84.5	175.1	53.7	6.42
PI 569295	88.7	307.3	74.5	17.55
PI 569520	84.3	287.3	73.6	16.89
PI 569590	90.9	318.4	70.7	23.26
PI 569597	87.7	226.6	53.3	19.82
PI 569644	86.2	290.7	64.3	17.85
PI 569670	84.4	303.1	78.6	17.33
PI 570717	102.4	262.8	58.0	27.90
PI 570718	84.3	207.6	62.6	20.00
PI 570731	87.9	287.6	64.8	31.93
PI 570747	112.9	318.4	61.9	41.42
PI 570753	83.0	193.0	60.6	15.48
PI 570759	89.8	76.0	61.4	7.63
PI 570761	120.0	423.8	56.9	52.28
PI 570775	90.0	235.8	63.8	21.05
PI 570877	75.2	204.3	56.8	6.88
PI 570957	138.1	389.1	63.7	36.69
PI 571067	81.0	313.3	76.0	17.68
PI 571068	83.2	189.2	68.3	11.50
PI 571073	110.7	350.3	60.8	24.27
PI 571120	76.9	317.3	71.5	18.53
PI 571126	93.6	335.6	67.9	33.00
PI 571176	122.8	393.1	65.8	20.81
PI 571276	78.5	281.1	69.8	26.06
PI 571284	98.8	299.4	70.4	15.05
PI 571370	.	356.0	70.2	28.57
PI 586791	111.1	375.8	70.0	42.54
NSL 50393	95.4	337.3	62.6	21.67
NSL 54316	82.0	250.8	62.8	22.97
NSL 55404	95.0	348.3	76.0	28.52
NSL 55429	91.0	253.9	62.6	22.79

Table 4. Cont'd

NSL 55431	100.2	323.5	52.2	33.20
NSL 55645	77.5	176.8	71.8	10.00
NSL 76942	147.2	303.7	67.6	25.92
NSL 82099	97.5	242.2	68.8	25.36
NSL 83601	93.8	328.8	65.2	27.70
NSL 83611	87.5	159.4	61.0	11.89
NSL 83656	.	311.0	71.9	31.91
NSL 83777	108.3	323.6	70.7	32.14
NSL 83779	99.6	301.7	62.9	30.84
NSL 83984	91.0	92.4	49.0	12.37
NSL 87920	91.4	317.5	56.2	32.78
NSL 92446	105.1	390.5	56.4	42.63
NSL 92465	97.2	176.3	59.8	23.73
NSL 103374	.	281.0	71.1	32.68
NSL 92465	88.0	104.3	58.9	14.79
NSL 92465	98.3	281.8	61.8	32.45
Green leaf	84.4	259.5	73.9	12.95
Roma	108.8	316.6	61.0	34.74
Theis	89.0	200.8	62.8	30.50
Dale	116.8	375.1	66.5	37.25
Wray	95.8	279.5	68.9	34.31
05CO9810(4)F3	98.8	180.6	66.2	19.68
Mall	83.0	270.1	67.5	21.14
SC-154	88.8	235.4	66.4	23.84
PMC-18	99.2	375.3	65.6	38.62
PMC-5	80.2	290.6	72.3	15.17
SC-163	90.5	224.3	67.9	12.13
SC-151	84.5	209.0	64.8	17.16
SC-161	79.8	280.1	69.6	18.96
SC-157	121.0	346.3	60.0	39.90
PSA-160	75.5	260.0	67.2	21.19
PMK-80	80.8	279.5	69.0	26.53
IS 19674	85.0	316.9	69.1	25.59
IS 9890	109.3	319.9	56.1	31.22
IS 22636	75.7	97.5	50.5	9.00
IS 20962	75.5	146.6	55.6	7.75
IS 21005	83.2	345.6	70.4	19.12
IS 21991	89.3	352.3	55.6	31.89
IS 21229	93.4	351.9	63.3	32.39
IS 21235	99.4	408.3	73.2	34.63
IS 20984	85.8	151.3	61.5	10.09
IS 21100	84.4	129.7	57.9	7.30
IS 21260	88.0	229.2	57.0	21.28
IS 131	89.5	105.3	61.6	5.89
IS 20888	78.3	256.0	69.5	16.90

Table 4. Cont'd

IS 20963	86.1	188.2	62.6	12.86
IS 20974	91.6	341.1	56.1	33.81
N98 short	88.8	267.2	57.7	33.94
N98 tall	116.0	395.7	68.8	25.92
N99	120.7	419.3	68.4	59.19
N100	.	212.5	75.8	11.30
N108	116.9	330.6	70.3	37.73
N104	83.1	188.9	71.2	20.44
N110	86.8	161.7	64.5	16.09
ICSR56	79.9	320.9	55.3	29.66
ICSR160	104.5	219.5	61.6	8.45
ICSR196	69.5	265.2	67.9	13.01
ICSR90017	80.8	247.6	68.5	14.79
ICSRP3034	85.9	309.1	71.6	15.20
ICSV700	99.1	304.5	64.6	28.11
S35	79.8	186.0	64.1	13.74
E36-1	93.3	346.7	60.7	38.93
NTJ2	86.5	218.6	68.8	19.66
Seredo	95.3	223.3	63.7	28.88
PI 154844	73.0	264.9	62.2	20.71
PI 175919	83.3	174.8	59.1	11.13
PI 217768	75.3	226.5	61.0	22.42
PI 22913	112.9	325.9	79.6	16.46
PI 248298	87.0	248.5	65.7	16.51
PI 257294	83.8	251.1	68.1	20.38
PI 257295	87.1	338.2	59.0	19.61
PI 562717	80.4	217.2	55.1	16.30
PI 571103	97.5	307.0	64.5	30.24
PI 591038	122.8	311.4	61.6	23.13
05C09880-1(2)	101.9	388.7	59.6	30.21
05C09881	93.8	379.2	60.0	29.20
05C09889-1-3	76.2	84.1	48.1	6.25
05C09890(1)	85.6	277.0	75.4	20.92
05C09892 (3) - 2	84.8	274.3	71.5	18.28
05C09654(3)	86.2	300.9	66.7	28.78
65D	81.8	245.1	65.9	21.50
Kanye standard	96.1	337.7	51.2	30.32
Marupantse	97.6	220.0	66.4	23.76
Mokula	83.9	297.6	67.9	25.32
Segaolane	84.1	337.6	67.5	20.18
Sureno	73.6	229.7	45.4	19.29

Table 4. Cont'd

05C09882(1)	76.8	350.9	68.7	25.69
05C09882(3)	91.5	290.1	80.6	19.00
05C09889(1)	98.8	322.8	69.7	31.61
05C09892(3)-4	84.5	325.1	77.5	24.24
05C09880(3)	102.1	351.5	63.4	46.57
05C09881(4)	.	234.5	71.7	18.89
05C09882(5)	94.8	225.8	67.1	23.58
05C09882(8)	95.2	323.7	62.7	23.09
05C09882(9)	90.0	272.1	66.9	24.58
05C09892(6)	101.5	269.2	60.6	26.69
05C09892(3)	87.2	160.8	49.2	16.93
05C09891(2)	71.8	248.1	62.9	13.66
SN372	80.5	257.1	63.8	24.70
Orange	100.8	407.3	63.9	53.80
Blackstrap	121.0	390.5	67.5	44.95
Mean	**92.4**	**275.5**	**64.6**	**24.20**
CV	**2.91**	**9.42**	**4.83**	**28.77**

Table 5. The Eigenvalues and principal components for anthesis date (AD), plant height (PH), moisture content (ML) and total biomass (DM) measured at Mead in 2009 showing the proportion explaining variation.

	Eigenvalue	Diff	Proportion	Cumulative
1	2.105	1.046	0.526	0.526
2	1.059	0.525	0.265	0.791
3	0.534	0.233	0.134	0.925
4	0.301		0.075	1

	Prin1	Prin2	Prin3	Prin4
Anthesis	0.540	-0.135	0.828	0.069
Height	0.602	0.172	-0.305	-0.717
Moist. Loss	0.131	0.938	0.041	0.317
Biomass Yield	0.573	-0.268	-0.468	0.616

Table 6. Polymorphic information content (PIC) of markers used to analyze 142 sorghum accessions.

Marker Type	Markers screened	Polymorphic markers	Number of bands	PIC		
				Min	Max	Mean
SRAP	49	40	109	0.145	0.939	0.557
SSR	33	29	84	0.221	0.75	0.519

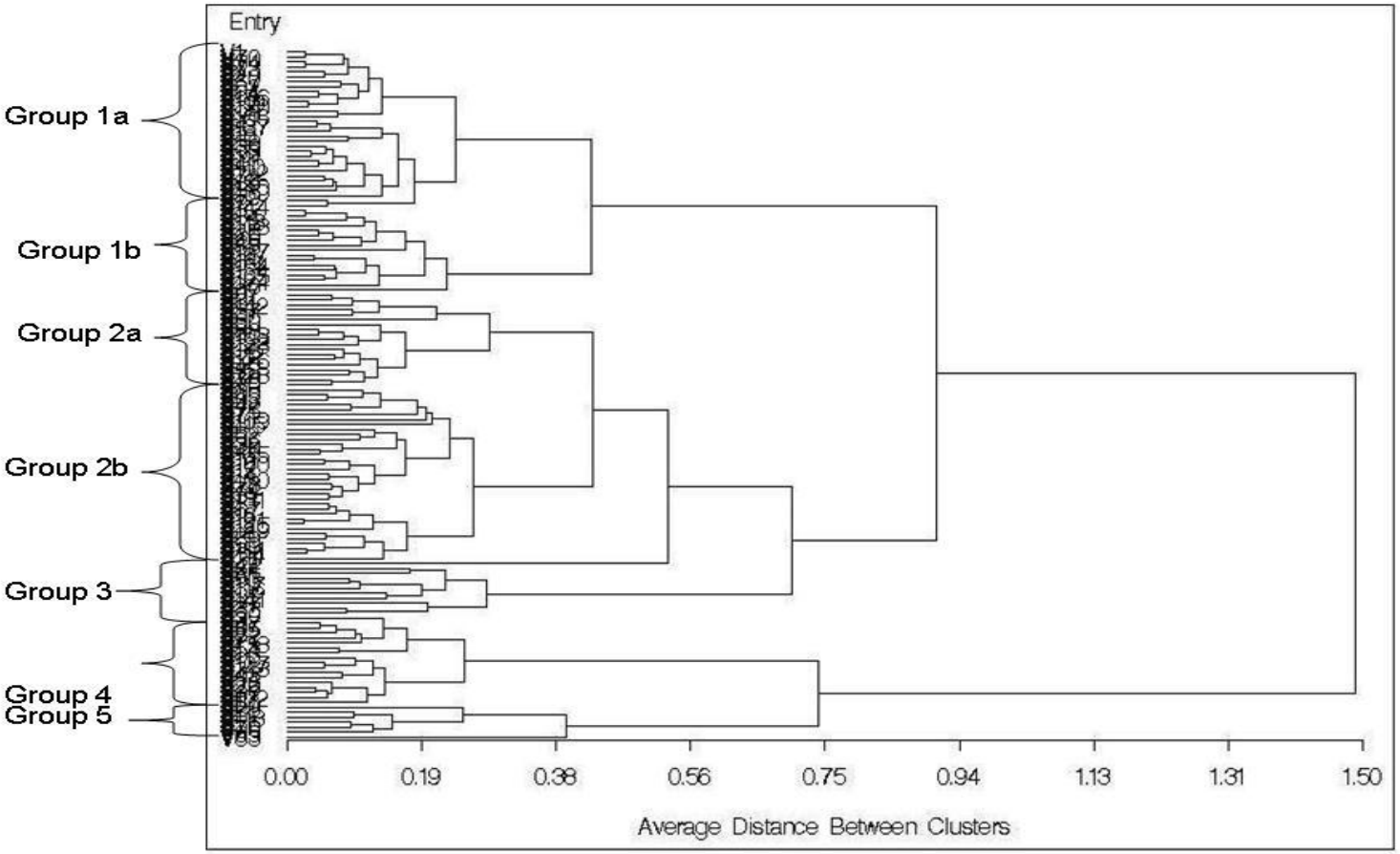


Figure 1. The dendrogram using average distances of 142 accessions based on anthesis date (AD), plant height (PH), moisture content (ML) and total biomass (DM) measured at Mead in 2009. Five major groups at threshold distance of 0.40.

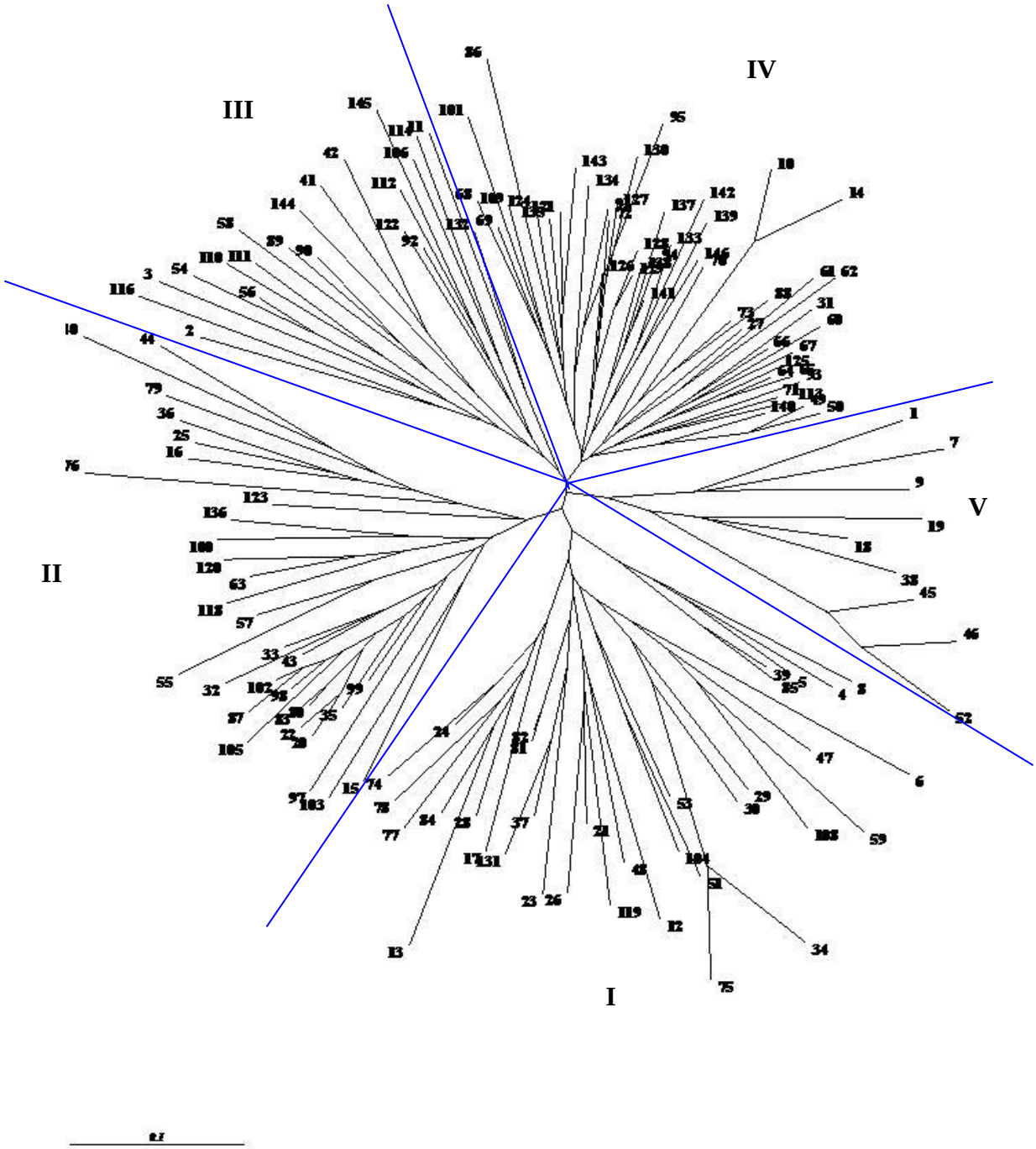


Figure 2. Dendrogram constructed by neighbor joining analysis using Nei's (1972) genetic standard distances of 142 sorghum accessions based on SSRs data

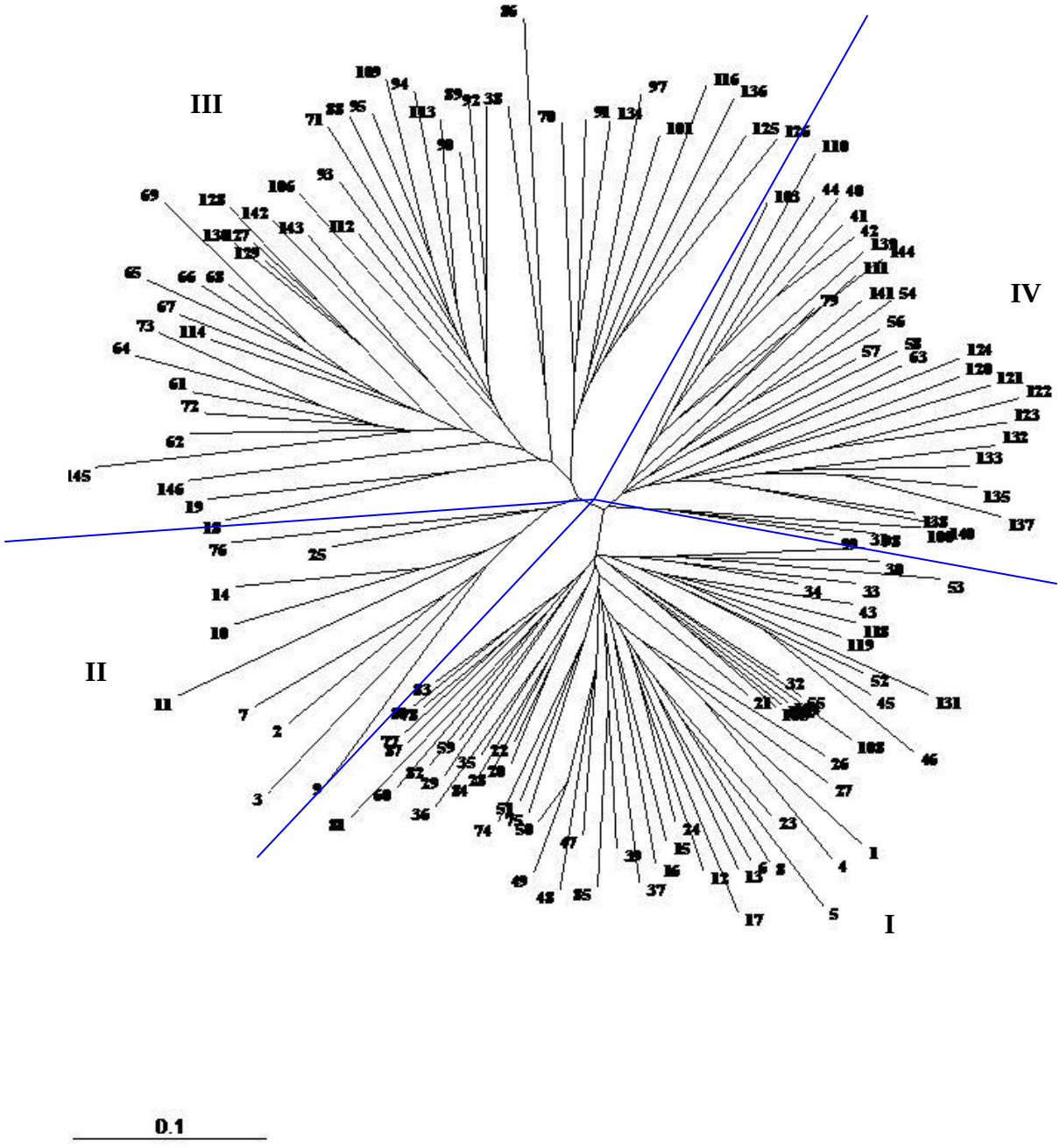


Figure 3. Dendrogram constructed by neighbor joining based on Nei's (1972) standard distances of 142 sorghum accessions based on SRAP data.

Appendices

Appendix 1. The analysis of variance for anthesis date (AD), plant height (PH), moisture content (MC) and total biomass (BY) of the 144 (including two check entries) accessions planted at Mead in 2009.

Anthesis date

Source	DF	SS	MS	F Value	P value
Replication	1	4.53	4.53	0.63	0.4285
Block(Rep)	22	216.1	9.82	1.37	0.1464
Entry	138	39008.91	282.67	39.36	<.0001
Residual	112	804.36	7.18	.	.

Plant height

Source	DF	SS	MS	F Value	P value
Replication	1	1961.99	1961.99	2.95	0.0888
Block(Rep)	22	25777.94	1171.73	1.76	0.0296
Entry	138	1289839.5	9346.66	14.04	<.0001
Residual	112	74575.05	665.85	.	.

Moisture loss

Source	DF	SS	MS	F Value	P value
Replication	1	122.45	122.45	12.84	0.0005
Block(Rep)	22	438.92	19.95	2.09	0.0065
Entry	138	9137.01	66.21	6.94	<.0001
Residual	112	1068.25	9.54	.	.

Dry matter yield

Source	DF	SS	MS	F Value	P value
Replication	1	9.87	9.87	0.21	0.6508
Block(Rep)	22	2199.62	99.98	2.09	0.0067
Entry	138	25708.15	186.29	3.89	<.0001
Residual	112	5367.2	47.92	.	.

Appendix 2. The cluster groups based on average distances of anthesis date (AD), plant height (PH), moisture content (MC) and total biomass (BY) planted at Mead in 2009.

Group 1					
Entry	**Label**	**Origin**	**Entry**	**Label**	**Origin**
V1	PI 217892	Sudan	V73	PMK-80	Botswana
V2	PI 246698	India	V84	IS 21260	Kenya
V4	PI 286245	India	V86	IS 20888	Angola
V7	PI 329761	Ethiopia	V89	N98 short	Nebraska
V10	PI 569154	Sudan	V97	ICSR160	ICRISAT
V15	PI 569597	Sudan	V98	ICSR196	ICRISAT
V18	PI 570717	Sudan	V99	ICSR90017	ICRISAT
V19	PI 570718	Sudan	V104	NTJ2	
V20	PI 570731	Sudan	V105	Seredo	Kenya
V25	PI 570775	Sudan	V106	PI 154844	Uganda
V26	PI 570877	Sudan	V108	PI 217768	Sudan
V34	PI 571276	Sudan	V110	PI 248298	India
V39	NSL 54316	Uganda	V111	PI 257294	Argentina
V41	NSL 55429	India	V113	PI 562717	Texas
V45	NSL 82099	Cameroon	V119	05C09890(1)	Nebraska
V57	NSL 92465	Ethiopia	V120	05C09892 (3) - 2	Nebraska
V58	Green leaf	Texas	V122	65D	Botswana
V62	Wray	Texas	V124	Marupantse	Botswana
V64	Mall	Botswana	V127	Sureno	Hunduras
V65	SC-154	Botswana	V134	05C09882(5)	Nebraska
V68	SC-163	Botswana	V136	05C09882(9)	Nebraska
V69	SC-151	Botswana	V137	05C09892(6)	Nebraska
V70	SC-161	Botswana	V139	05C09891(2)	Nebraska
V72	PSA-160	Botswana	V140	SN372	Texas

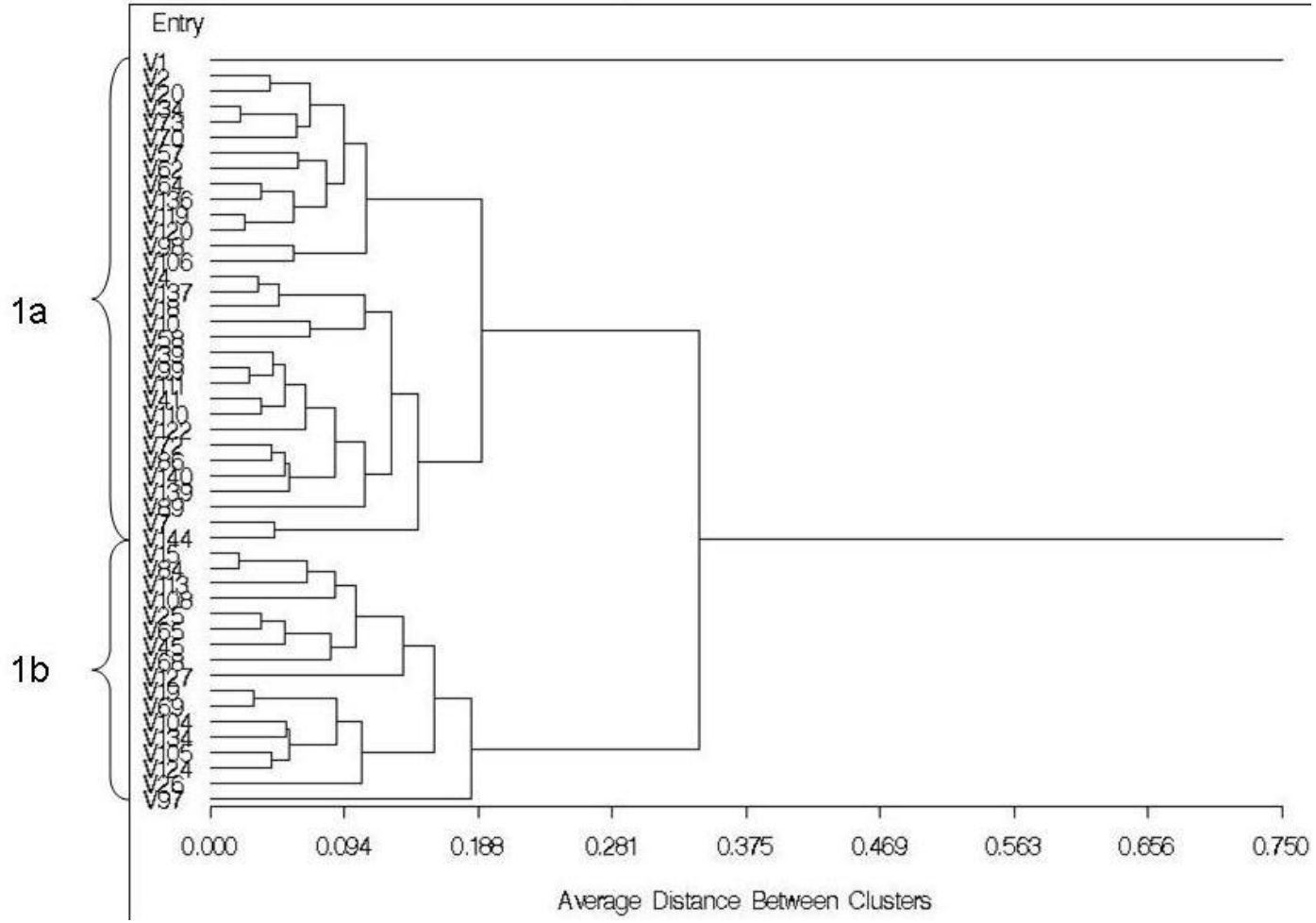
Group 2					
Entry	**Label**	**Origin**	**Entry**	**Label**	**Origin**
V5	PI 287611	Zimbabwe	V71	SC-157	Botswana
V6	PI 329336	Ethiopia	V74	IS 19674	Zimbabwe
V8	PI 562943	Sudan	V75	IS 9890	Sudan
V9	PI 569009	Sudan	V78	IS 21005	Kenya
V12	PI 569295	Sudan	V79	IS 21991	India
V13	PI 569520	Sudan	V80	IS 21229	Kenya
V14	PI 569590	Sudan	V88	IS 20974	Kenya
V16	PI 569644	Sudan	V93	N108	Nebraska
V17	PI 569670	Sudan	V96	ICSR56	ICRISAT
V21	PI 570747	Sudan	V100	ICSRP3034	ICRISAT
V28	PI 571067	Sudan	V101	ICSV700	ICRISAT
V30	PI 571073	Sudan	V103	E36-1	Ethiopia
V31	PI 571120	Sudan	V109	PI 22913	China
V32	PI 571126	Sudan	V112	PI 257295	Argentina

Appendix 2. Cont'd

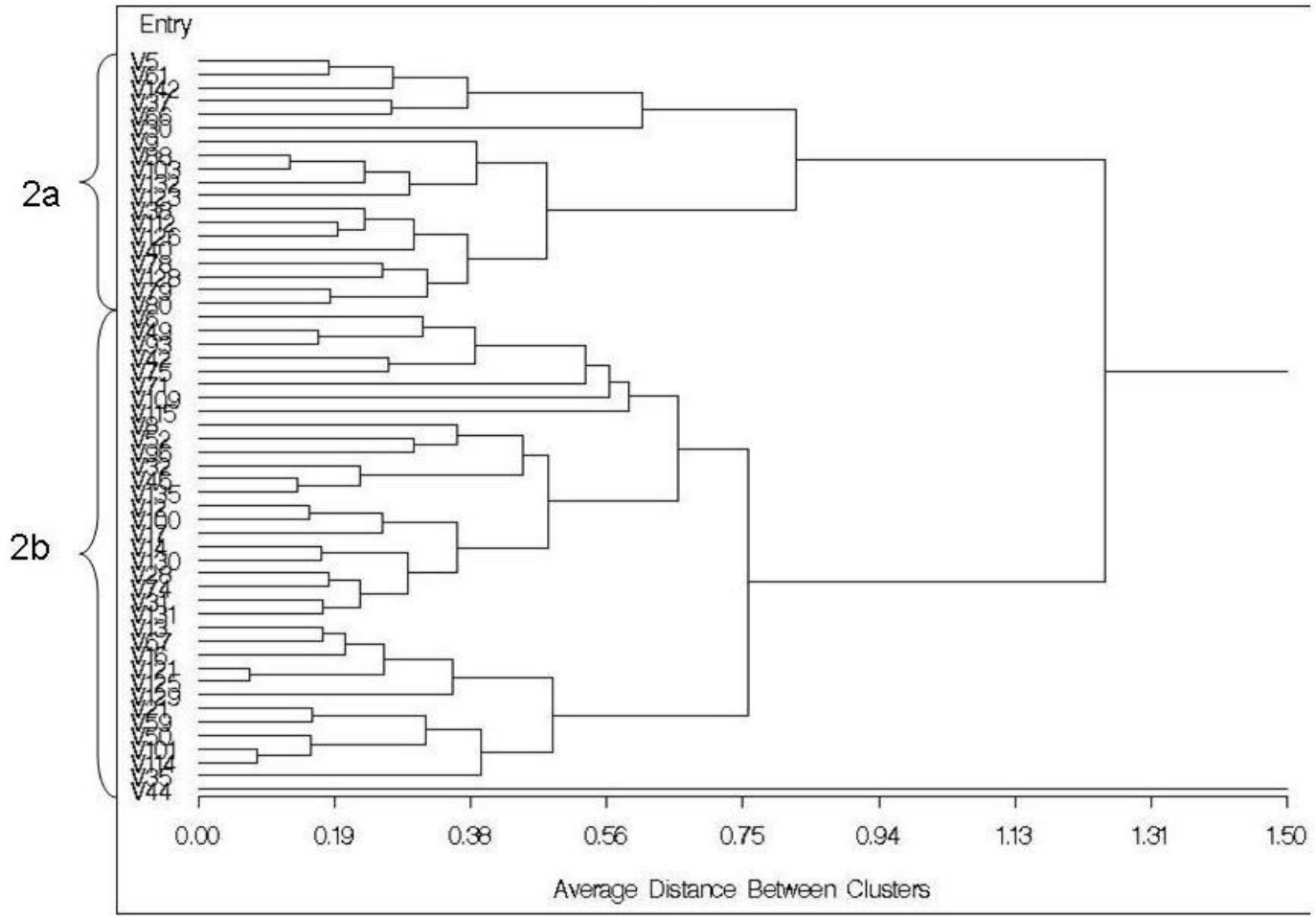
V35	PI 571284	Sudan	V114	PI 571103	Sudan
V37	PI 586791	Sudan	V115	PI 591038	Nigeria
V38	NSL 50393	Indiana	V121	05C09654(3)	Nebraska
V40	NSL 55404	India	V123	Kanye standard	Botswana
V42	NSL 55431	India	V125	Mokula	Botswana
V44	NSL 76942	India	V126	Segaolane	Botswana
V46	NSL 83601	Cameroon	V128	05C09882(1)	Nebraska
V49	NSL 83777	Cameroon	V129	05C09882(3)	Nebraska
V50	NSL 83779	Cameroon	V130	05C09889(1)	Nebraska
V52	NSL 87920	Cameroon	V131	05C09892(3)-4	Nebraska
V59	Roma	South Africa	V132	05C09880(3)	Nebraska
V61	Dale	Mississippi	V135	05C09882(8)	Nebraska
V66	PMC-18	Botswana	V142	Blackstrap	Kansas
V67	PMC-5	Botswana			

Group 3			**Group 4**		
Entry	**Label**	**Origin**	**Entry**	**Label**	**Origin**
V24	PI 570761	Sudan	V3	PI 276804	Ethiopia
V81	IS 21235	Kenya	V47	NSL 83611	Cameroon
V53	NSL 92446	Ethiopia	V95	N110	Kansas
V117	05C09881	Nebraska	V82	IS 20984	Kenya
V116	05C09880-1(2)	Nebraska	V77	IS 20962	Kenya
V91	N99	Nebraska	V138	05C09892(3)	Nebraska
V141	Orange		V54	NSL 92465	Ethiopia
V27	PI 570957	Sudan	V63	05CO9810(4)F3	Nebraska
V33	PI 571176	Sudan	V11	PI 569283	Sudan
V90	N98 tall	Nebraska	V107	PI 175919	Maryland
			V143	Sugar Drip	
	Group 5		V43		
Entry	**Label**	**Origin**	V94	N104	Oklahoma
V23	PI 570759	Sudan	V22	PI 570753	Sudan
V51	NSL 83984	Cameroon	V29	PI 571068	Sudan
V118	05C09889-1-3	Nebraska	V87	IS 20963	Kenya
V56	NSL 92465	Ethiopia	V102	S35	Nigeria
V76	IS 22636	Cameroon	V60	Theis	Mississippi
V85	IS 131	Ohio			
V83	IS 21100	Kenya			

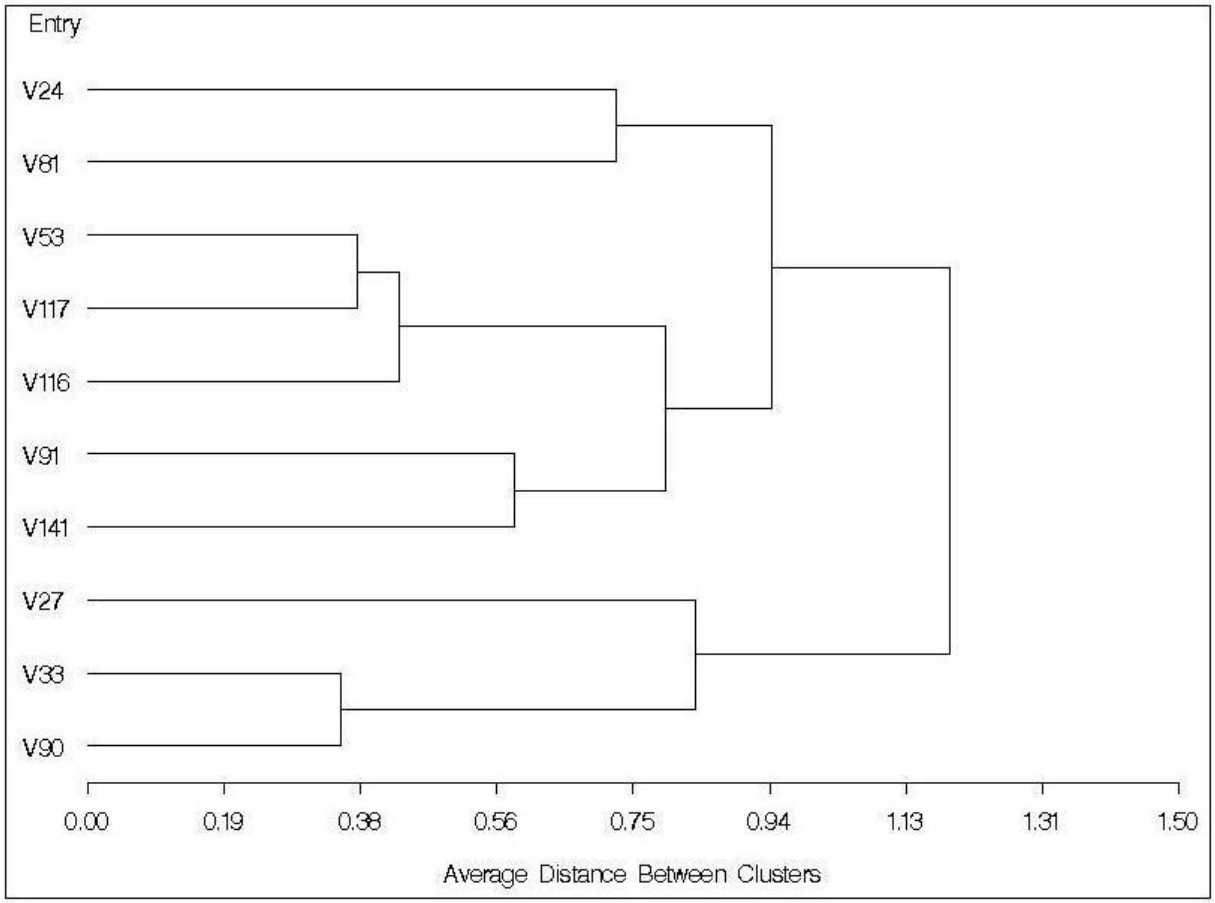
Appendix 3. Dendrogram showing the accessions that clustered into group 1 by average distance based on four agronomic traits.



Appendix 4. Dendrogram showing the accessions that clustered into group 2 by average distance based on four agronomic traits



Appendix 5. Dendrogram showing the accessions that clustered into group 3 by average distance based on four agronomic traits



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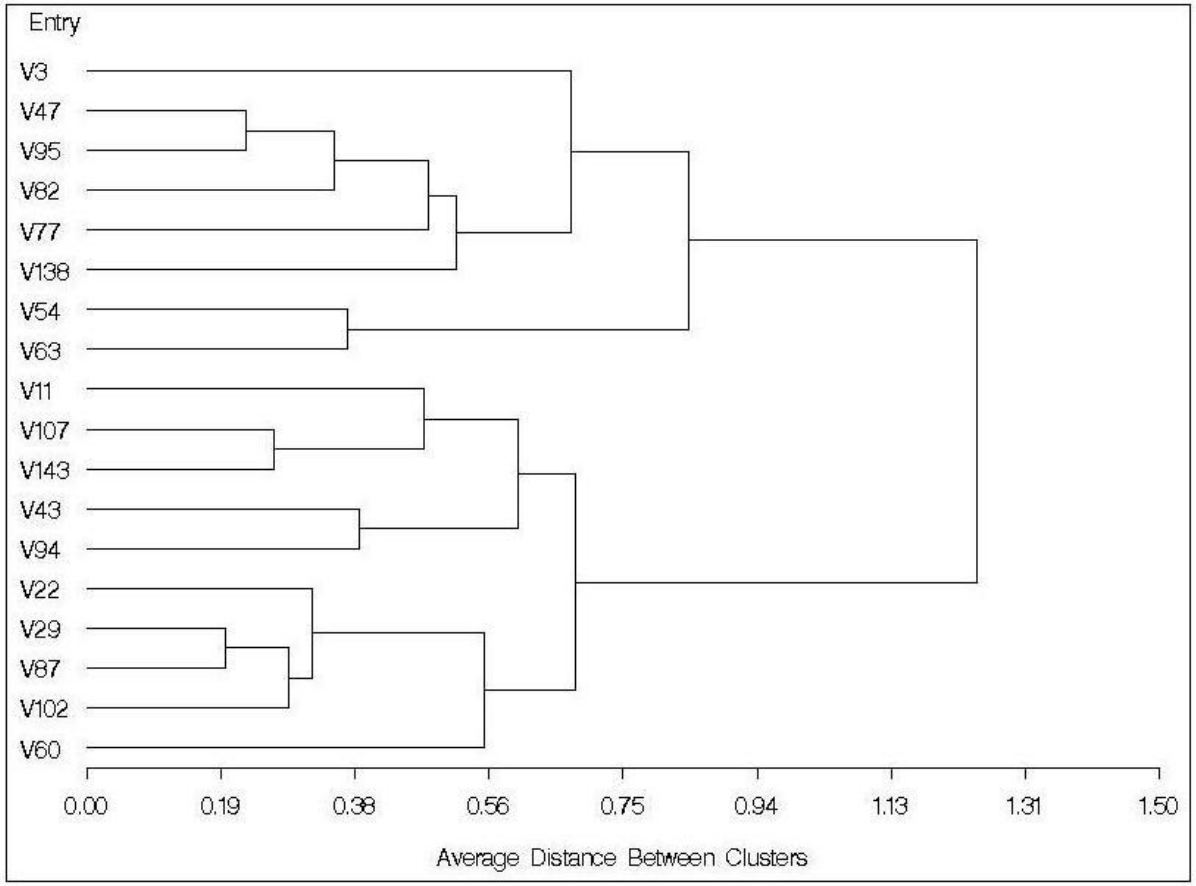
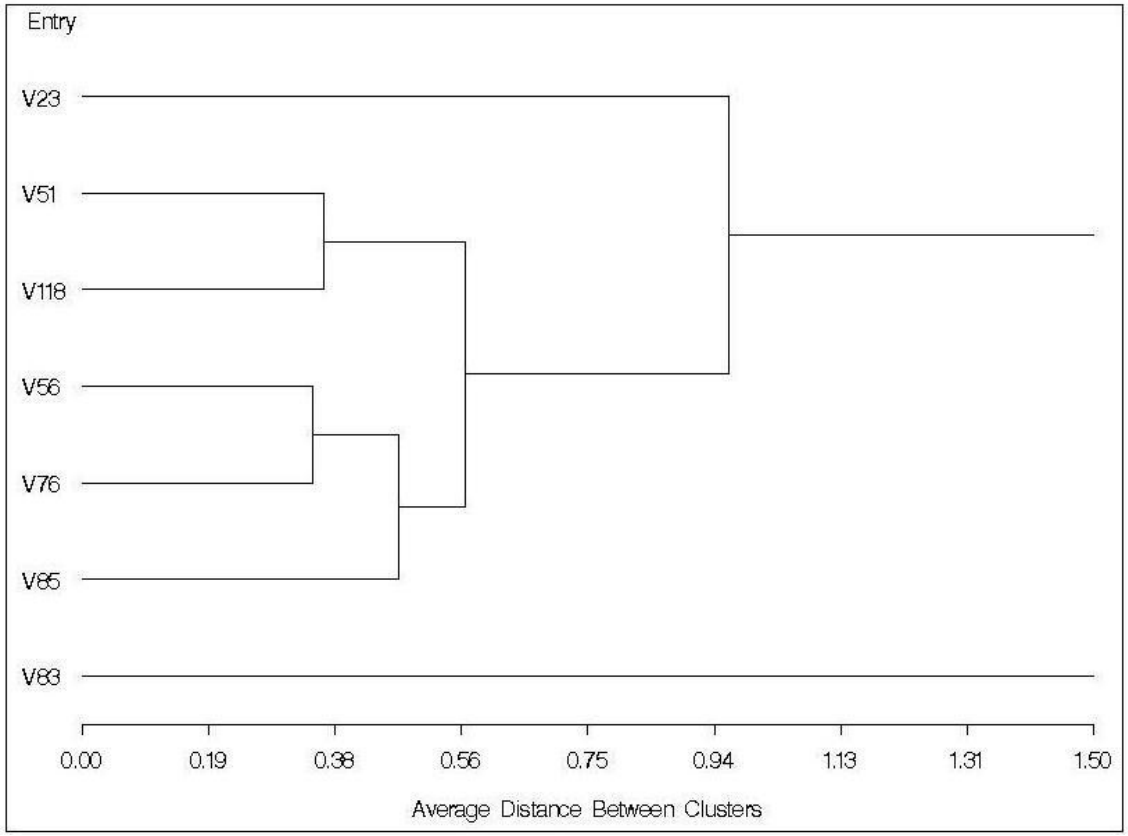
Appendix 6. Dendrogram showing the accessions that clustered into group 4 by average distance based on four agronomic traits.



Appendix 7. Dendrogram showing the accessions that clustered into group 5 by average distance based on four agronomic traits.



Appendix 8. The polymorphic information content (PIC) of individual markers used to assess the diversity of 142 sorghum accessions.

SRAP combination	No. of alleles	PIC
SRAP01	4	0.747
SRAP02	4	0.696
SRAP03	1	0.625
SRAP04	2	0.375
SRAP05	1	0.750
SRAP06	3	0.364
SRAP07	6	0.801
SRAP08	1	0.939
SRAP10	2	0.470
SRAP11	3	0.658
SRAP12	2	0.493
SRAP13	2	0.500
SRAP14	2	0.383
SRAP16	4	0.746
SRAP17	2	0.365
SRAP19	2	0.162
SRAP20	1	0.939
SRAP21	2	0.349
SRAP22	2	0.499
SRAP25	4	0.639
SRAP26	2	0.417
SRAP27	2	0.145
SRAP28	3	0.211
SRAP29	4	0.660
SRAP30	3	0.591
SRAP31	1	0.839
SRAP32	2	0.252
SRAP34	2	0.490
SRAP35	9	0.859
SRAP36	3	0.664
SRAP37	3	0.612
SRAP38	2	0.377
SRAP39	3	0.634
SRAP41	2	0.487
SRAP42	5	0.591
SRAP43	1	0.389
SRAP46	4	0.692
SRAP47	2	0.431
SRAP48	1	0.667
SRAP49	5	0.759
Min	**1**	**0.145**
Max	**9 (109)†**	**0.939**
Mean	**2.73**	**0.557**

SSR Marker Name	No. of alleles	PIC
LBK22	4	0.741
LBK41	4	0.750
Xcup01	4	0.702
xcup02	2	0.488
Xcup05	3	0.645
Xcup07	2	0.321
Xcup13	3	0.478
Xcup14	2	0.454
Xcup16	2	0.392
Xcup20	2	0.341
Xcup23	2	0.221
Xcup26	2	0.411
Xcup28	3	0.584
Xcup36	2	0.488
Xcup38	2	0.464
Xcup43	2	0.500
Xcup47	2	0.460
Xcup48	4	0.715
Xcup49	5	0.696
Xcup50	3	0.616
Xcup53	6	0.645
Xcup57	3	0.549
Xcup61	4	0.604
Xcup63	2	0.276
Xcup64	3	0.541
Xcup67	2	0.462
Xcup69	4	0.598
Xcup73	3	0.632
Xcup74	2	0.281
Min	**2**	**0.221**
Max	**6 (84)†**	**0.750**
Mean	**2.90**	**0.519**

†- number in brackets indicates the total number of polymorphic alleles used for cluster analysis

Appendix 9. The cluster groups of 142 sorghum accessions analyzed by neighbor joining using Nei's (1972) genetic standard distance based on SSRs data.

Entry	Accession number/name	Origin	Entry	Accession number/name	Origin
	Group 1			**Group 2**	
8	PI 562943	Sudan	15	PI 569597	Sudan
4	PI 286245	India	103	S35	Nigeria
5	PI 287611	Zimbabwe	97	ICSR56	ICRISAT
39	NSL 54316	Uganda	99	ICSR196	ICRISAT
85	IS 131	Ohio, USA	35	PI 571284	Sudan
6	PI 329336	Ethiopia	20	PI 570731	Sudan
47	NSL 83611	Cameroon	22	PI 570753	Sudan
59	Roma	South Africa	80	IS 21229	Kenya
108	PI 154844 01 SD	Uganda	83	IS 21100	Kenya
29	PI 571068	Sudan	105	NTJ2	
30	PI 571073	Sudan	98	ICSR160	ICRISAT
34	PI 571276	Sudan	87	IS 20963	Kenya
75	IS 9890	Sudan	102	ICSV700	ICRISAT
53	NSL 92446	Ethiopia	43	NSL 55645	Uganda
51	NSL 83984	Cameroon	32	PI 571126	Sudan
104	E36-1	Ethiopia	33	PI 571176	Sudan
12	PI 569295	Sudan	55	NSL 103374	Cameroon
48	NSL 83656	Cameroon	57	NSL 92465 (Red)	Ethiopia
119	PI 591038 01 SD	Nigeria	118	PI 571103 01 SD	Sudan
21	PI 570747	Sudan	63	05CO9810 (4) F3	Nebraska
26	PI 570877	Sudan	120	05C09880-1(2)	Nebraska
23	PI 570759	Sudan	100	ICSR90017	ICRISAT
37	PI 586791	Sudan	136	05C09880(3)tan	Nebraska
131	Sureno	Hunduras	123	05C09890(1) PP bmr	Nebraska
81	IS 21235	Kenya	76	IS 22636	Cameroon
82	IS 20984	Kenya	16	PI 569644	Sudan
17	PI 569670	Sudan	25	PI 570775	Sudan
28	PI 571067	Sudan	36	PI 571370	Sudan
13	PI 569520	Sudan	79	IS 21991	India
84	IS 21260	Kenya	40	NSL 55404	India
77	IS 20962	Kenya	44	NSL 76942	India
78	IS 21005	Kenya			
24	PI 570761	Sudan			
74	IS 19674	Zimbabwe			

Appendix 9. Cont'd

	Group 4	
68	SC - 163	Botswana
69	SC - 15	Botswana
101	ICSRP3034	ICRISAT
109	PI 175919 01 SD	Maryland
86	IS 20888	Angola
124	05C09892 (3) - 2 tanmedbmr	Nebraska
135	05C09892(3)-4 tallbmr	Nebraska
121	05C09881msTAN	Nebraska
143	05C09891(2) bmr	Nebraska
134	05C09889(1) vtallsw	Nebraska
91	N99	Nebraska
95	Norkan	Kansas
126	65D	Botswana
72	PSA - 160	Botswana
127	Kanye standard	Botswana
130	Segaolane	Botswana
128	Marupantse	Botswana
129	Mokula	Botswana
138	05C09882(5) tan	Nebraska
137	05C09881(4)ppbmr	Nebraska
94	Wheatland	Oklahoma
141	05C09892(6) ppbmrsw	Nebraska
133	05C09882(3) tanbmr	Nebraska
142	05C09892(3) tanbmr	Nebraska
139	05C09882(8) tanbmr	Nebraska
146	Blackstrap	Kansas
70	SC - 161	Botswana
10	PI 569154	Sudan
14	PI 569590	Sudan
73	PMK - 80	Botswana
88	IS 20974	Kenya
27	PI 570957	Sudan
61	Dale	Mississippi
62	Cowley	Texas
31	PI 571120	Sudan
66	PMC - 18	Botswana
60	Theis	Mississippi
67	PMC - 5	Botswana
125	05C09654(3) sw	Nebraska
64	Mall	Botswana
65	SC - 154	Botswana
93	N108	Nebraska
71	SC - 157	Botswana
113	PI 257294 02 SD	Argentina
140	05C09882(9) tanOP	Nebraska
49	NSL 83777	Cameroon
50	NSL 83779	Cameroon

	Group 3	
2	PI 246698	India
116	PI 562717 01 SD	Texas
3	PI 276804	Ethiopia
54	NSL 92465 (Orange-red)	Ethiopia
56	NSL 92465 (White)	Ethiopia
110	PI 217768 02 SD	Sudan
111	PI 22913 04 SD	China
58	Green leaf	Texas
89	N98 short	Nebraska
90	N98 tall	Nebraska
144	SN372 - Chinese Amber	Texas
41	NSL 55429	India
42	NSL 55431	India
122	05C09889-1-3tall tan	Nebraska
92	N100	Nebraska
112	PI 248298 01 SD	India
145	Orange	
106	Seredo	Kenya
114	PI 257295 03 SD	Argentina
11	PI 569283	Sudan
132	05C09882(1)tanbmr	Nebraska

	Group 5	
1	PI 217892	Sudan
7	PI 329761	Ethiopia
9	PI 569009	Sudan
19	PI 570718	Sudan
18	PI 570717	Sudan
38	NSL 50393 (PI 651101)	Indiana
45	NSL 82099	Cameroon
46	NSL 83601	Cameroon
52	NSL 87920	Cameroon

Appendix 10. List of sequence-related amplified polymorphism (SRAP) marker combinations and sequences used in analyzing 142 sorghum accessions.

ID	Sequence
Forward	
Me1	TGA GTC CAA ACC GGA TA
Me2	TGA GTC CAA ACC GGA GC
Me3	TGA GTC CAA ACC GGA AT
Me4	TGA GTC CAA ACC GGA CC
Me5	TGA GTC CAA ACC GGA AG
Me6	TGA GTC CAA ACC GGA CA
Me7	TGA GTC CAA ACC GGA CG
Reverse	
Em1	GAC TGC GTA CGA ATT AAT
Em2	GAC TGC GTA CGA ATT TGC
Em3	GAC TGC GTA CGA ATT GAC
Em4	GAC TGC GTA CGA ATT TGA
Em5	GAC TGC GTA CGA ATT AAC
Em6	GAC TGC GTA CGA ATT GCA
Em7	GAC TGC GTA CGA ATT CAA

SRAP combination	Forward	Reverse
SRAP01	Me1	Em1
SRAP02	Me1	Em2
SRAP03	Me1	Em3
SRAP04	Me1	Em4
SRAP05	Me1	Em5
SRAP06	Me1	Em6
SRAP07	Me1	Em7
SRAP08	Me2	Em1
SRAP09	Me2	Em2
SRAP10	Me2	Em3
SRAP11	Me2	Em4
SRAP12	Me2	Em5
SRAP13	Me2	Em6
SRAP14	Me2	Em7
SRAP15	Me3	Em1
SRAP16	Me3	Em2
SRAP17	Me3	Em3
SRAP18	Me3	Em4
SRAP19	Me3	Em5
SRAP20	Me3	Em6
SRAP21	Me3	Em7
SRAP22	Me4	Em1
SRAP23	Me4	Em2
SRAP24	Me4	Em3
SRAP25	Me4	Em4
SRAP26	Me4	Em5
SRAP27	Me4	Em6
SRAP28	Me4	Em7
SRAP29	Me5	Em1
SRAP30	Me5	Em2
SRAP31	Me5	Em3
SRAP32	Me5	Em4
SRAP33	Me5	Em5
SRAP34	Me5	Em6
SRAP35	Me5	Em7
SRAP36	Me6	Em1
SRAP37	Me6	Em2
SRAP38	Me6	Em3
SRAP39	Me6	Em4
SRAP40	Me6	Em5
SRAP41	Me6	Em6
SRAP42	Me6	Em7
SRAP43	Me7	Em1
SRAP44	Me7	Em2
SRAP45	Me7	Em3
SRAP46	Me7	Em4
SRAP47	Me7	Em5
SRAP48	Me7	Em6
SRAP49	Me7	Em7

Appendix 11. . The cluster groups of 142 sorghum germplasm accessions analyzed by neighbor joining using Nei's (1972) genetic standard distance based on SRAPs data

	Group 1	
31	PI 571120	Sudan
30	PI 571073	Sudan
53	NSL 92446	Ethiopia
33	PI 571176	Sudan
34	PI 571276	Sudan
43	NSL 55645	Uganda
118	PI 571103 01 SD	Sudan
119	PI 591038 01 SD	Nigeria
131	Sureno	Hunduras
52	NSL 87920	Cameroon
45	NSL 82099	Cameroon
46	NSL 83601	Cameroon
32	PI 571126	Sudan
55	NSL 103374	Cameroon
108	PI 154844 01 SD	Uganda
104	E36-1	Ethiopia
102	ICSV700	ICRISAT
105	NTJ2	
21	PI 570747	Sudan
26	PI 570877	Sudan
27	PI 570957	Sudan
1	PI 217892	Sudan
4	PI 286245	India
23	PI 570759	Sudan
5	PI 287611	Zimbabwe
8	PI 562943	Sudan
6	PI 329336	Ethiopia
13	PI 569520	Sudan
17	PI 569670	Sudan
12	PI 569295	Sudan
24	PI 570761	Sudan
15	PI 569597	Sudan
16	PI 569644	Sudan
37	PI 586791	Sudan
39	NSL 54316	Uganda
85	IS 131	Ohio
47	NSL 83611	Cameroon
48	NSL 83656	Cameroon
49	NSL 83777	Cameroon
50	NSL 83779	Cameroon
75	IS 9890	Sudan
51	NSL 83984	Cameroon
74	IS 19674	Zimbabwe
20	PI 570731	Sudan
22	PI 570753	Sudan
28	PI 571067	Sudan
84	IS 21260	Kenya
35	PI 571284	Sudan
36	PI 571370	Sudan
29	PI 571068	Sudan
59	Roma	South Africa
82	IS 20984	Kenya
60	Theis	Mississippi
81	IS 21235	Kenya
87	IS 20963	Kenya
77	IS 20962	Kenya
78	IS 21005	Kenya
80	IS 21229	Kenya
83	IS 21100	Kenya

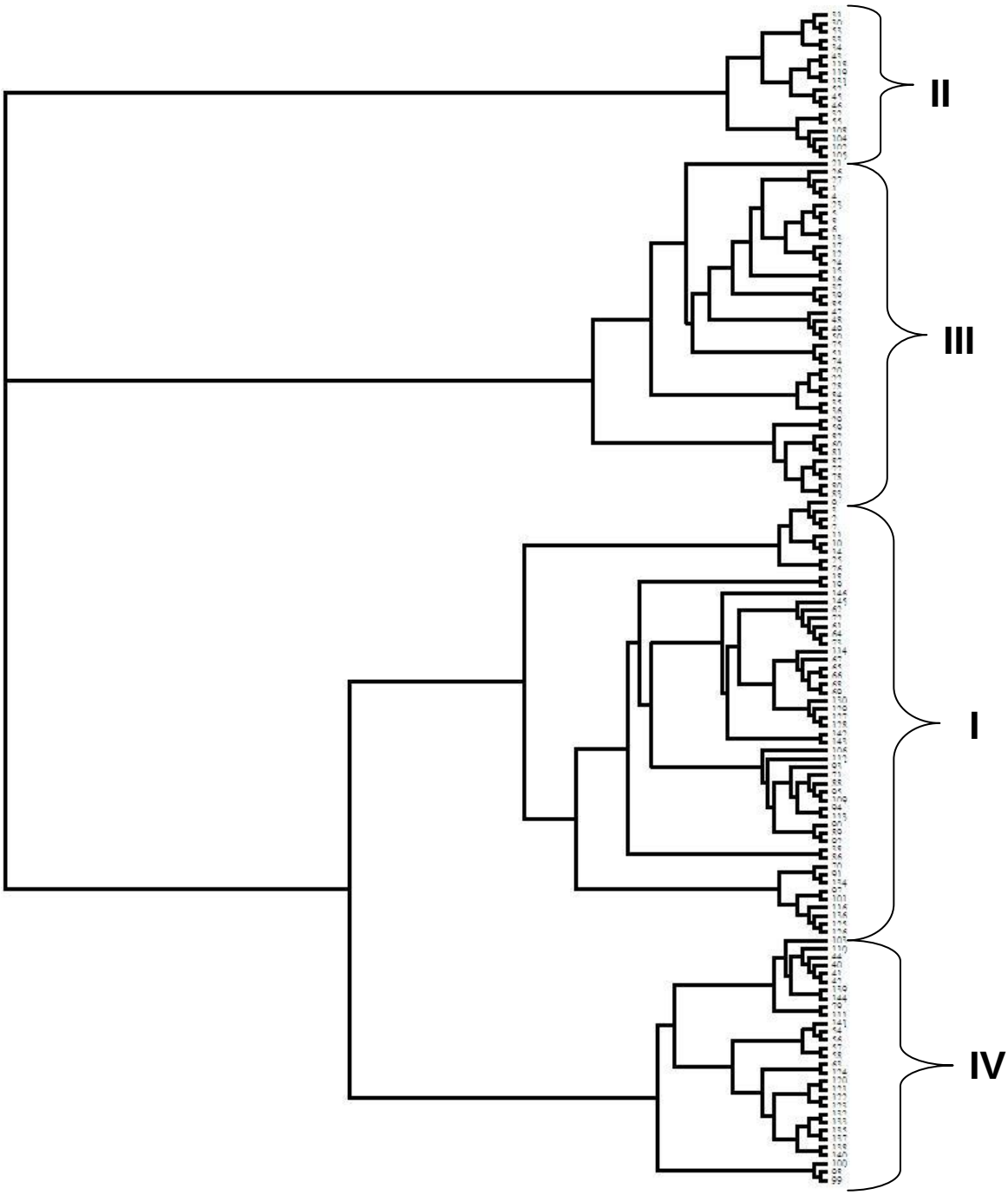
	Group 2	
9	PI 569009	Sudan
3	PI 276804	Ethiopia
2	PI 246698	India
7	PI 329761	Ethiopia
11	PI 569283	Sudan
10	PI 569154	Sudan
14	PI 569590	Sudan
25	PI 570775	Sudan
76	IS 22636	Cameroon

Appendix 11. Cont'd

Group 3		
18	PI 570717	Sudan
19	PI 570718	Sudan
146	Blackstrap	Kansas
145	Orange	
62	Cowley	Texas
72	PSA - 160	Botswana
61	Dale	Mississippi
64	Mall	Botswana
73	PMK - 80	Botswana
114	PI 257295 03 SD	Argentina
67	PMC - 5	Botswana
65	SC - 154	Botswana
66	PMC - 18	Botswana
68	SC - 163	Botswana
69	SC - 15	Botswana
130	Segaolane	Botswana
129	Mokula	Botswana
127	Kanye standard	Botswana
128	Marupantse	Botswana
142	05C09892(3) tanbmr	Nebraska
143	05C09891(2) bmr	Nebraska
106	Seredo	Kenya
112	PI 248298 01 SD	India
93	N108	Nebraska
71	SC - 157	Botswana
88	IS 20974	Kenya
95	Norkan	Kansas
109	PI 175919 01 SD	Maryland
94	Wheatland	Oklahoma
113	PI 257294 02 SD	Argentina
90	N98 tall	Nebraska
89	N98 short	Nebraska
92	N100	Nebraska
38	NSL 50393 (PI 651101)	Indiana
86	IS 20888	Angola
70	SC - 161	Botswana
91	N99	Nebraska
134	05C09889(1) vtallsw	Nebraska
97	ICSR56	ICRISAT
101	ICSRP3034	ICRISAT
116	PI 562717 01 SD	Texas
136	05C09880(3)tan	Nebraska
125	05C09654(3) sw	Nebraska
126	65D	Botswana

Group 4		
103	S35	Nigeria
110	PI 217768 02 SD	Sudan
44	NSL 76942	India
40	NSL 55404	India
41	NSL 55429	India
42	NSL 55431	India
139	05C09882(8) tanbmr	Nebraska
144	SN372 - Chinese Amber	Texas
79	IS 21991	India
111	PI 22913 04 SD	China
141	05C09892(6) ppbmrsw	Nebraska
54	NSL 92465 (Orange-red)	Ethiopia
56	NSL 92465 (White)	Ethiopia
57	NSL 92465 (Red)	Ethiopia
58	Green leaf	Texas
63	05CO9810 (4) F3	Nebraska
124	05C09892 (3) - 2 tanmedbmr	Nebraska
120	05C09880-1(2)	Nebraska
121	05C09881msTAN	Nebraska
122	05C09889-1-3tall tan	Nebraska
123	05C09890(1) PP bmr	Nebraska
132	05C09882(1)tanbmr	Nebraska
133	05C09882(3) tanbmr	Nebraska
135	05C09892(3)-4 tallbmr	Nebraska
137	05C09881(4)ppbmr	Nebraska
138	05C09882(5) tan	Nebraska
140	05C09882(9) tanOP	Nebraska
100	ICSR90017	ICRISAT
98	ICSR160	ICRISAT
99	ICSR196	ICRISAT

Appendix 12. Dendrogram constructed by neighbor joining based on Nei's (1972) genetic standard distances of 142 sorghum accessions using SRAPs data.



Chapter 2

GENETIC MAPPING OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH BIOENERGY TRAITS IN SWEET SORGHUM

Genetic mapping of quantitative trait loci associated with bioenergy traits in sweet sorghum

Abstract

Sorghum is tropical C4 photosynthesis that is able to thrive in marginal and harsh environmental conditions, making it to be considered a poor man's crop. The agronomics of sweet sorghum (drought tolerance, wide adaptation and high sugar content) make sweet sorghum an ideal crop for cellulosic biofuel production. Therefore mapping of bioenergy traits in sweet sorghum could help in narrowing the search for the genes responsible for those trait variations. Recombinant inbred lines (RILs) from two sorghum lines were screened using SSR markers, and four environments for anthesis date (AD), plant height (PH), moisture content (MC), total biomass yield (BY), and brix in Nebraska. The RILs were significantly different ($P<0.05$) for all the traits, and showed normality necessary for QTL analysis. The RILs showed transgressive segregation for all the traits, which was consistent with other sweet sorghum mapping studies. The genetic map constructed from 158 SSRs spanned a length of 1541.3 cM, and generated 18 linkage groups that corresponded to the 10 sorghum chromosomes. Fourteen QTLs (6 for brix, 3 for BY, 2 each for AD and MC, and 1 for PH) were mapped for the four and the combined environments. The brix QTLs mapped on linkage groups (LG) 1b, 4b, 5 and 7 explaining 6.4 to 33.9% of phenotypic variation, while BY QTLs mapped on LG 1b, 9b and 10b and explained 9.7 to 17.4% of phenotypic variation. The AD QTLs mapped on LG 1b and explained variation of 26.2 to 58.4%, MC QTLs mapped on LG 1b and 6a, and explained 7.7 to 77.0% variation, while PH QTL mapped on LG 7 and explained

14.7% variation. Traits that showed significant correlation colocalized on LG 1b suggesting that chromosome 1 might be of significant importance in selecting lines for improved bioenergy.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a tropical C4 photosynthesis plant using complex biochemical and morphological specializations to improve carbon assimilation at high temperatures (Paterson, 2008). Sorghum thrives in marginal and harsh environmental conditions, and thus is considered a poor man's crop. Sorghum is preferentially grown in water-limited environments both in developed and developing countries (Mace et al., 2009). Though there have been some improvements in grain and forage sorghum, the amount of resources invested in the crop has been minimal compare to other species (Saballos, 2008). In addition to sorghum's importance as a food crop, it is also considered an important crop in molecular genetics/biology as a model for complex grass genomes like that of sugarcane and maize. The small genome of sorghum has long been an attractive model for advancing our understanding of the structure, function and evolution of cereal genomes, and its genome has lower levels of gene duplications compared to other tropical cereals (Paterson, 2008). Sweet sorghum, another variety of sorghum has shown a high level of synteny with sugarcane (Grivet et al., 1994; Ming et al., 1998). Breeding strategies have been adopted for the construction of linkage maps and identify genomic regions associated with traits of importance. Sweet sorghum has gained momentum as a potential bioenergy crop because of its high biomass yield, and high sugar content in its juicy stems. In addition, the advanced level of knowledge regarding genetic control of perenniality in sorghum contributes to its promise as a bioenergy crop (Paterson, 2008).

The agronomics of sweet sorghum (drought tolerance, wide adaptation and high sugar content) make sweet sorghum to be an ideal crop for biofuel production. Sweet

sorghum has the ability to accumulate 10 – 25% sugar in the stalk juice near the time of maturity (Ritter et al., 2007). At least one report indicates that sweet sorghum was used for sugar and alcohol production since the 17th century (Vaidyanathan et al., 1987), although its usage was limited because of other sugar crops like sugarcane that were in more advanced stage in their industrial development. Since the sugar content in the stalk is mainly sucrose (Vaidyanathan et al., 1987; Ritter et al., 2007), this makes it easy to ferment (Ali et al., 2008). As a bioenergy crop, sweet sorghum could be used to provide grain starch which has more or less the same value as corn starch for ethanol production; stem juice for direct fermentation; and bagasse left after juice extraction as lignocellulosic feedstock for fermentation or boiler fuel (Saballos, 2008). However, there are limited studies regarding the genetics of sorghum carbohydrates and biomass production. Several studies have stipulated that the sucrose phosphate synthase (SPS) is one of the enzymes associated with sucrose concentration in plants (Bruneau et. al., 1991; McIntyre et al., 2006; Grof et al., 2006), and the SPS family is also linked to several important agronomic traits. Therefore mapping of bioenergy traits in sweet sorghum could help in narrowing the search for the genes responsible for those traits variation.

The availability of molecular marker maps has made it possible to map loci that control quantitative traits. Genetic linkage mapping in sorghum is made easy by its straightforward diploid genetics, high levels of polymorphisms, and the ability to easily carry out inbreeding (Paterson, 2008). Genetic linkage maps are essential for studying the inheritance of traits, map-based cloning, and for comparative genomics (Mace et al., 2009). Although genes have been identified that control some agronomic traits (e.g. Patterson et al., 1995; Pereira et al., 1995; Tao et al., 2000; Klein et al., 2001; Tao et al.,

2003; Parh et al., 2006), there are still more genes or genomic regions that control quantitative characters of importance that still need to be mapped (Hart et al, 2001). Several QTLs have been identified in sorghum like disease resistance (Gowda et al., 1995; Tao et al., 2003; Parh et al., 2006), drought resistance (Tuinstra et al., 1996), insect resistance (Nagaraj et al., 2005), staygreen (Harris et al., 2007), fertility restoration (Klein et al., 2005), photoperiod sensitivity (Crasta et al., 1999; Chantereau et al., 2001), and plant height and maturity (Pereira et al., 1995). QTL analysis is important in evaluating the inheritance and the feasibility of accelerating gains from selection for complex quantitative traits in crops (Ejeta et al., 1999). Most of the bioenergy associated traits like biomass, carbohydrates, and stem juiciness are complex as shown by their distribution in sorghum populations. Several studies and research experiments have shown these traits to exhibit a continuous variation in a population, indicating that there are several genes responsible for the observed variability. Saballos (2008) stated that the most important parameter in ethanol production is total soluble carbohydrate yield.

The total soluble carbohydrate concentration and biomass yield will be a major factor in ethanol production potential of an individual genotype. The main carbohydrate in sorghum stem juice is sucrose, with variable amounts of reducing sugars and starch (Saballos, 2008), and its concentration and composition differ between genotypes. The mechanism of sugar accumulation in sweet sorghum has been shown to differ from that of sugarcane as shown by enzymatic control and carbon transport (Lingle, 1987; Tarpley and Vietor, 2007; Murray et al., 2009). Stem sugar concentration inheritance is not simple; environment, genetic, genetic x environment interaction, and epistasis (genetic background) all play a role (Murray et al., 2009). Stem sugar concentration QTLs have

been reported to explain little phenotypic variation because of its moderate heritabilities (0.51 – 0.86) (Schlehuber, 1945; Natoli et al., 2002; Brian et al., 2006; Ritter et al., 2008; Murray et al., 2008a). There is little known about the genetics and importance of other traits like moisture content in sorghum. Moisture content of the stem plays a major role in what variety is considered juicy or not. Saballos (2008) cited Swanson and Parker (1931) who reported a single recessive gene controlling juicy stems to a dominant dry/pithy stems. However, the observed continuous variation in the amount of extractible juice in juicy genotypes or the offspring of the crosses between juicy and pithy or juicy by juicy lines suggest that more genes are involved in controlling the trait. Therefore the objectives of this study were:

1. To identify genomic regions (QTLs) associated with bioenergy related traits on a SSRs sorghum linkage map
2. To determine the relationship between bioenergy traits and other agronomic traits

Materials and methods

Plant material

One hundred and sixty five (165) $F_{6:7}$ recombinant inbred lines (RILs) were developed from a cross between 09178(1) and N99 and were used for the QTLs evaluation in this study. The female parent, 09178(1) is a genetic male sterile F_3 line derived from a cross between 122*ms*$_3$ and wheatland and has the *bmr12* gene (brown mid-rib). 122*ms*$_3$ is a NPSS selection (Nebraska population) developed by Dr. J. Eastin, that possesses the male sterile loci *ms*$_3$. Therefore, this line still may have some loci segregating for other traits. 09178(1) is considered a non-sweet (grain sorghum type) line

with approximate brix of 6, and as a female parent is shorter and earlier flowering than N99. A single gene male sterility results from homozygosity at one of the six *ms* loci, and ms_3 has been used extensively in sorghum breeding programs because of its stability over a range of environments (Rai et al., 1999; Saballos, 2008). The male parent, N99 is a Nebraska line released in 1990 derived from a cross between Fremont and Theis (Gorz et. al., 1990) that is considered to be a good forage line with brix of 19. The cross between 09178(1) and N99 was made because of the contrasting differences in plant height and brix reading. The $F_{6:7}$ RILs were derived through single seed descent procedure by continuous selfing of individuals in each generation.

Agronomic traits

Field experiments and data

The 165 RILs, the parental lines and two check cultivars (Segaolane & Simon) were planted under rainfed conditions at Havelock and at Mead during the 2008 and 2009 seasons (May 15th and 16th 2008; and May 14th and 16th 2009 at Havelock and Mead respectively). Recombinant inbred lines were planted on conventionally tilled plots. At Havelock, the lines were planted in a field following soybean (*Glycine max* (L.) Merr.) in soybean-sorghum rotation with no artificial fertilizer application, while at Mead 91.2 Kg ha^{-1} of anhydrous ammonia fertilizer was applied each year. The two check cultivars were included in each incomplete block. The experiment design was an alpha lattice incomplete block design with 13 incomplete blocks of 15 plots each (13 x 15 alpha lattice) per replication, with two replications at each environment. Single row plots

measuring five meters long with 0.75m between rows were sown at the rate of 50 seeds per row.

Five agronomic traits were measured, that included anthesis date measured as the duration in days from planting to 50% of the plants within a plot were shedding pollen; plant height measured as the distance from the base of the plant to the tip of the panicle; total biomass in Mg ha^{-1} when plants had reached their physiological maturity; moisture content as the percentage difference between wet biomass and dried biomass weight; Brix degree (1^{o} brix) as a measure of soluble solids (mainly sucrose) in the stem juice. To obtain total biomass, a random sample from a 2 m plot length was obtained by cutting the plants at near the soil surface and weighed immediately to obtain the wet weight, then a random subsample of five plants was weighed, and then separated into panicles (heads), leaves and stems. The subsamples were then bagged and placed into an oven at 120 – 160^{o}C for ten days to completely dry the samples. Samples were then reweighed to obtain the dry weight. Total biomass was calculated as follows:

$$BY\ (Mg/ha) = \frac{Subsample\ dry\ weight\ (g)}{Subsample\ wet\ weigh\ (g)} X \frac{2\ m\ plot\ wet\ weight\ (g)}{plot\ area\ (m2)\ x\ 150}$$

whereby a factor of 150 converts grams per plot to Mg ha^{-1} ((g/1.50m^2)*(10000m^2/ha)*(Mg/100 000g)). Brix reading was obtained from the five stems of the subsamples before drying using a hand-held refractometer (MASTER-T Brix 0.0-32.0% with ATC, Atago Co., LTD, Tokyo, Japan).

DNA extraction and Marker analysis

Genomic DNA of each accession was extracted from fresh leaf tissues from plants planted in the greenhouse using cetyltrimethyl ammonium bromide (CTAB) protocol (Dweikat, 2005; Mahmood, 2004). The ground tissue was incubated in extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 1 M NaCl, 1% CTAB, 1mM 1,10-phenathroline and 0.15% 2-mercaptoethanol) at 65^{o}C for 1 hr; then equal volume of chloroform:isoamyl alcohol (24:1) added to the tissue mixture. After centrifugation at 3000 rpm, the supernatant was transferred to a new clean tube and DNA was precipitated with equal volume of cold isopropanol. DNA was air died at room temperature for an hour and then re-suspended in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) with 20ng RNase and incubated at 37^{o}C overnight. Equal volume of 24:1 Chloroform:isoamyl alcohol was added to the DNA-RNase mix and centrifuged at 3000 rpm for 5 minutes, the resulting supernatant was transferred to new tube. Two volumes of cold absolute ethanol and 5µl of 8M ammonium acetate were added to the supernatant in order to precipitate the DNA. After centrifugation, DNA pellets were air dried at room temperature, and later re-suspended with 200 - 400 µl TE buffer depending on the size of the pellet. DNA concentration was determined using a spectrophotometer (TKO 100 Fluorometer, Hoefer Scientific Instruments, San Francisco, CA).

A collection of 1003 SSR sorghum oligonucleotide primer pairs (Brown et al., 1996; Taramino et al., 1997; Bhattramakki et al., 2000; Kong et al., 2000; Schloss et al. 2002; Lubbock, TX unpublished; ICRISAT; Burrow et al., 2008; Srinivas et al., 2008; Srinivas et al., 2009; Li et al., 2009) were synthesized, and marker assays were conducted following the procedure of Kuleung et al. (2004). A 25 µl total/reaction was used

consisting of 75 ng genomic DNA, 100 ng primer pair, 125 μM dNTP, 50 mM KCl and 10 mM Tris-HCl, 2mM $MgCl_2$, and 1 unit Taq polymerase. The amplification procedure consisted of one cycle at 94°C for 3 min. followed by 35 cycles of 1 min at 94°C, 1 min at 55 to 58°C depending on the primer pair, 1 min at 72°C, and final extension step at 72°C for 5 min. The reaction was then cooled to a resting temperature of 4°C and resolved by electrophoresis in a 12% non-denatured polyacrylamide gels (37:1 acrylamide:bis-acrylamide). The gels were stained in 1 ug/ml ethidium bromide for 10 min, distained in deionized water for 15 min; then, photographed using the Gel Doc2000 (Bio-Rad, Hercules, CA).

Phenotypic data analysis

Analysis of variance (ANOVA) for anthesis date, plant height, moisture content, total biomass, and Brix was performed for each individual environment using the PROC MIXED procedure (Littell et al., 1996) of SAS version 9.2 (SAS Institute, 2008) where lines were considered as fixed effects, and replications and blocks as random effects. Prior to combined analysis, homogeneity of error variances was checked with Bartlett's Chi-square test as outlined by Gomez and Gomez (1984). In the combined analysis, lines were considered as fixed, and environments (location-year), blocks within environments, and genotype x environment interaction (GEI) were considered as random. Narrow-sense entry mean heritabilities with standard errors were estimated for the mapping population using the PROC MIXED procedure of SAS version 9.2. For the heritabilities estimates, parents and checks data were excluded, and estimates followed a method described by Holland et al. (2003). The basic SAS code for heritability estimate is available at

http://www4.ncsu.edu/~jholland/heritability/Inbreds.html (Verified on 02 Feb 2010). Pearson's correlation coefficients between traits were calculated for the combined least square genotypic means using the PROC CORR procedure of SAS. The RIL trait data were subjected to normality test using PROC UNIVARIATE, Q-Q plots and P-P plots to determine its suitability for QTL analysis. A total of 1003 SSR markers were screened for polymorphisms between the two parents. Markers that showed polymorphisms between the two parents were then used to screen the RIL population. The parental amplified DNA samples were included as controls with every set of 26 lines to facilitate scoring. Prior to map construction, all polymorphic markers used for screening the population were checked by the chi-square ($\chi 2$) test for the goodness of fit against a 1:1 segregation ratio at the 0.05 probability level.

Map construction

MAPMAKER/ EXP3.0 program (Lander et al. 1987) was used for marker diagnostics to determine the linkage groups. MAPMAKER performs full multipoint linkage analyses (simultaneous estimation of all recombination fractions from the primary data). The linkage groups identified were considered not linked if the distance between flanking markers was greater than 37.2 cM and the logarithm of odds (LOD) score was not less than 3. Map distances (in Centi-Morgan units) were calculated using the Kosambi mapping function (Kosambi, 1944). Co-segregating markers (defined as mapping within a 0.2 cM interval) were excluded from the final map and only one marker for each cluster was retained.

QTL analysis

QTL analysis was performed with the composite interval mapping (CIM) (Zeng 1994) method of WinQTL cartographer v.2.5 (Wang et al., 2007; http://statgen.ncsu.edu/qtlcart/WQTLCart.htm, verified on 05 Feb. 2010) using the least squares means of each trait for each of the four environments and the mean of the combined environments. The threshold for declaring the presence of a significant QTL for each trait–environment combination was defined by 1000 permutations at $P \leq 0.05$ (Churchill and Doerge, 1994) in order to handle non-normality in both the marker and the trait data. The walking speed chosen for all traits was 1 cM. Cofactors were determined following the standard CIM model, using the forward and backward regression method with a probability in and out of 0.1. The position where the logarithm of odds (LOD) score curve reaches its maximum was used as the estimate of the QTL location. The value of the additive effect (a) at each QTL peak LOD score positions was computed as half of the difference between the mean phenotypic values of the two groups of the RILs based on the information of the flanking markers and with the assumption that all lines were homozygous for one or the other of the parental alleles at that QTL region. The percentage of the phenotypic variance explained by a QTL was estimated as the coefficient of determination (R^2) using single-factor analysis from a general linear models procedure (Wang et al., 2007). For each QTL, R^2 was determined for the single marker closest to the identified QTL. The QTLs detected above the LOD threshold that explained more than 10% of the variance in at least one environment were arbitrarily classified as major QTLs and those explaining less than 10% as minor QTLs. A 95% confidence interval was established by marking ±1 LOD score marker positions,

following Lander and Botstein's (1989) 'LOD drop-off method'. Quantitative trait loci for the same trait detected at different environments were considered to be the same if the estimated map position of their peaks fell within 20 cM of each other.

Results and discussion

Phenotypic data

Harvest began approximately four weeks after the last anthesis date was recorded, and the duration of harvest ranged from six to eight weeks depending on the weather and the speed of sample processing especially for brix reading. Brix from each incomplete block was obtained within 24 hours after harvest, and the samples were kept in the cold room to minimize further breakdown of sugars in the stem juice. The experimental design (alpha lattice design with incomplete blocks) made it possible to adjust for the errors due to harvest, because each incomplete block was harvested within a reasonable time. Phenotypes of the RILs showed significant differences ($P<0.05$) for all the traits measured within each environment (Appendix 3 – 6). These significant differences within a single environment indicate the significance of genotypic/genetic effect in this mapping population. Studies have shown that total biomass is positively correlated with flowering dates, as documented in this study too that the late flowering lines accumulating higher biomass (Table 2). The RILs showed transgressive segregation for all the traits except plant height, and in most cases the mean value of the traits was intermediate of the parental lines (Table 1). Transgressive segregation can be caused by both parental lines contributing favorable or unfavorable alleles for a particular trait and is common in inbred populations (Wang and Goldman, 1997; Rosenberg et al, 1999; Rosenberg et al,

2003), or breakage of linkage between favorable and unfavorable allele, as well as failure to statistically declare small QTLs. The normality tests showed that the data was suitable for QTL analysis in all the environments and for all traits (data not shown). The normal distribution within each individual environment signified the continuous genetic variations that exist between the RILs.

The evidence of environmental variation could also be observed because traits exhibited high significant difference ($P<0.05$) for the environments in the combined analysis (Appendix 2), and the mean ranges differed between the 2008 and 2009 seasons at both locations (Table 1). The 2009 season at Mead had slightly higher trait values compared to the other environments, and the 2008 season at Mead had the lowest trait means for all the traits except anthesis date (AD) and brix. Variation between environments is caused by both biotic and abiotic factors (Murray et al., 2008a), and these factors have substantial impact on agronomic traits of crops that leads to highly significant GxE interaction (GEI). There was a high amount of trait variation between and among environments for the RILs (significant differences among lines at $P<0.05$) (Appendix 2 - 6), with AD ranging from 61days at Mead in 2009 to 103 days at Havelock in 2008; plant height (PH) from 144.4 cm at Havelock in 2009 to 318.8 cm at Mead in 2008; moisture content (MC) from 41.5% at Mead in 2009 to 75.6% at Mead in 2009; total biomass yield (BY) from 5.39 Mg ha^{-1} at Mead 2009 to 27.28 Mg ha^{-1} at Mead in 2009; and brix from 6.6 at Mead in 2009 to 23.7 at Mead in 2009 (Table 1). There was a significant positive correlation between AD with MC and BY; PH with all other traits; and BY with brix values (Table 2). These traits were also highly correlated at each environment (Appendix 7). There was no significant correlation between brix values and

MC. Considering that brix measures the amount of solids in the stem juice, it was expected to find a significant correlation between the two traits. Murray et al. (2008a,b) reported as expected a significant correlation between brix values and stem juiciness (moisture content). Late maturing lines had higher total biomass yield with high moisture content, and as expected plant height also contributed to a higher biomass yield. Taller plants also have greater stem harvest index compared to shorter ones. The heritability values estimated for all the environments varied among traits and ranged from 0.16 (SE = 0.11) for total biomass yield for the combined environments to 0.98 (SE=0.003) for anthesis date at Mead in 2008 (Table 1). High heritabilities for flowering date, plant height and brix values in sorghum have been reported by other researchers (Brown et al., 2006; Ritter et al., 2008; Murray et al., 2008a,b). The high heritabilities for anthesis date and plant height support the reason why the two traits are of importance in selection for most breeding programs.

Linkage map construction

Out of the 1003 SSR markers screened with the parental lines of the RIL population, 278 markers were polymorphic. Of the 278 markers, 208 were produced polymorphisms with the RILs and were scored. The other 70 markers were highly distorted, mostly skewed towards N99, and were not used; 22 markers showed unclear polymorphisms and were excluded in order to minimize scoring errors, and only 186 markers were used in the linkage map construction. The *Xsbarslbk* designated markers (generated from cDNA library) were most affected by the distortion compared to other markers. The 186 markers used for linkage map (LG) construction did not significantly

deviate from 1:1 segregation ratio. The final map contained 158 markers that were distributed on 18 linkage groups spanning a length of 1541.3 cM (Appendix 1). The other 28 markers could not be mapped to any linkage group. This gave an average marker distance of 9.8 cM. The linkage map constructed here is most likely the first sorghum genetic linkage map constructed entirely of SSR markers. The available sorghum genetic linkage maps are based mainly on RFLPs or a combination of different markers types especially RFLPs with other marker types such as SSRs (Bhattramakki et al., 2000; Menz et al., 2002; Schloss et al., 2002; Bowers et al., 2003), AFLPs, RAPDs (Haussmann et al., 2002), DArTs (Mace et al., 2009) just to name a few. Even those maps that are said to be SSR based (Taramino et al., 1997; Kong et al., 2000), combine in part some RFLP framework linkage map. The total map length was within the previously reported range, for example Mace et al. (2009) reported a map length of 1603.5 cM for a consensus map using SSRs, DArT and RFLPs; and Murray et al. (2008a) reported even a larger length of 1836 cM. All the linkage groups could be assigned to the ten chromosomes location based on the positioning of the commonly mapped SSRs like the *Xtxtp*, *Xcup* and *Xgap* markers (Menz et. al, 2002; Srinivas et. al., 2009), and the LG nomenclature followed chromosome naming suggested by Kim et al. (2005). The linkage groups ranged from 4.8 cM (Sbi08b) with two markers to 281.7 cM (Sbi01b) with 24 markers. Sbi07 was the most densely populated linkage group with 12 markers for a length of 92.5 cM. The marker order was in good colinearity with previously published linkage maps (Murray et al., 2008a; Srinivas et al, 2008; Burrow et al., 2009; Mace et al., 2009; Srinivas et al, 2009). The few differences in marker order were mainly for closely spaced markers, and this has been observed in other studies. Mace et al. (2009) stated that marker

rearrangement could be due to error in small population sizes or statistical uncertainty of orders in data sets, and in cases where markers are truly non-conforming to previously mapped location can be a result of mapping paralogous loci.

QTL analysis

The composite interval mapping (CIM) identified some QTLs in the sorghum genome that were associated with most traits measured. Approximate QTL locations for all environments are presented in Figure 1, and the exact positions are shown in Table 3. A total of 14 QTLs were detected using CIM (2-LOD intervals) for all the six measured traits with 6 for brix, 2 for total biomass, 2 each for anthesis date and moisture content, and one for plant height (Table 3; Fig. 1). The QTLs for total biomass and brix were detected in three out of the four environments, while there were no QTLs detected for Mead location in 2009. The inability to detect QTLs on some chromosomes/linkage group indicates the significance of population background and environmental effects on QTL analysis. Due to the highly significant GEI exhibited by the phenotypic data, not all locations show QTL effects. In addition to the GEI, population size, trait heritability, and recombination affect the ability to accurately detect QTLs (Collard et al., 2005). Quantitative trait loci colocalization clusters were observed on linkage group Sbi01b. These corresponded to anthesis date, brix, and moisture contents QTLs. The brix QTLs mapped towards the proximal end of the linkage group Sbi01b colocalized with total biomass. Colocalization may suggest pleiotropy where a genomic region contains genes that affects a number of traits or several genes linked but each affecting a different trait (Ritter et al., 2008). Brix was highly correlated to total biomass (Table 2; Appendix 7).

There were six QTLs associated with brix and detected on linkage group Sbi01b, Sbi04b, Sbi05, and Sbi07. Murray et al. (2008a,b) also reported QTLs for brix on the sorghum chromosome Sbi01, Sbi03, and Sbi07. This study did not detect any brix QTL on chromosome Sbi03 as reported by other authors (Brown et. al., 2006; Ritter et. al., 2008; Murray et. al., 2008a,b). Murray et al. (2009) also reported that there was no brix QTL association on chromosome Sbi03 in their association study. The largest QTL, Brix_01 explained 33.9 % of the phenotypic variation and was placed near the marker locus *Tx145* at position 56.7 cM on LG Sbi01b. It was detected at Havelock in 2008 only, and the 09178(1) allele decreased brix value by 6.7 (Table 3). Brix_02 explained 10.8% of the phenotypic variation, and was placed near the marker loci *Xtxtp343* at position 32.9 cM on LG Sbi04b. It was detected at Mead in 2008 and combined environments, and the 09178(1) allele decreased brix values by approximately 1 (Table 3). QTLs Brix_03, Brix_04 and Brix_06 explained 7.8, 8.4 and 6.6% of the phenotypic variation respectively, and were placed near the marker loci *Xcup28*, *Xsbarslbk7.63* and *Xsbarslbk5.08* at positions 52.7 (LG Sbi04b), 87.1 (LG Sbi07) and 94.2 cM (LG Sbi05) respectively. All those QTLs, 09178(1) allele also decreased brix values (Table 3). The last brix QTL, Brix_05 explained 6.4% of the phenotypic variation and was placed near the marker locus *Tx147* at position 189.4 cM on LG Sbi01b. This N99 allele increased the brix values by 0.7 (table 3).

Three QTLs for total biomass were detected on the linkage group Sbi01b, Sbi09b, and Sbi10b. Ritter et al. (2008) also mapped total biomass QTLs on three sorghum chromosomes Sbi01, Sbi06 and Sbi10. The largest QTL, BY_01 explained 17.4% of the phenotypic variation and was placed near the marker locus *sam38725* at

position 26.2 cM on LG Sbi09b. It was detected only at Mead in 2008, and the 09178(1) allele decreased total biomass by 1.23 Mg ha^{-1}. BY_02 explained 13.3% of the phenotypic variation, and was placed near the marker loci *Xsbarslbk1.61* at position 205.6 cM on LG Sbi01b. It was detected at Havelock and Mead in 2008, and the N99 allele increased total biomass by 1.16 Mg ha^{-1}. The third total biomass QTL, BY_03 explained 9.7% of the phenotypic variation and was placed near the marker locus *Xcup16* at position 18.3 cM on LG Sbi10b. The 09178(1) allele decreased the total biomass by 1.19 Mg ha^{-1} (Table 3).

The QTLs for the anthesis date were detected at two regions on the linkage group Sbi01b. Natoli et. al. (2002) also reported flowering days QTLs on sorghum chromosomes Sbi01 and Sbi05. The largest QTL, AD_01 explained 58.4% of the phenotypic variation and was placed near marker locus *Drenhsbm63* at position 27.0 cM. It was detected at Mead in 2008 only, and the 09178(1) allele decreased flowering time by 29 days. AD_02 explained 26.2% phenotypic variation, and was placed near the marker loci *Xtxtp284* at position 167.4 cM. It was detected at Havelock in 2009, and the 09178(1) allele decreased flowering time by 19 days (Table 3). Plant height QTL, PH_01 was detected on the linkage group Sbi07 at Havelock in 2008 only. The 09178(1) allele decreased height by 16.7 cm (Table 3). Previous researches have also mapped QTL for height on sorghum chromosome Sbi07 (Multani et. al., 2003; Ritter et. al., 2008; Murray et. al., 2008a), and this region is also associated with the dw_3 dwarfness allele. PH_01 explained 14.7% of the phenotypic variation and was placed near marker locus *Xsbarslbk7.59* at position 55.0 cM (Table 3).

The QTLs for moisture content were detected on linkage group Sbi01b and Sbi06a. The largest QTL, MC_01 explained 77.0% of phenotypic variation and was placed near the marker locus *Tx145* at position 52.6 cM on LG Sbi01b. It was detected at Havelock in 2008 only, and the 09178(1) allele decreased moisture content by 30.5% (Table 3). MC_02 explained 7.7% of the phenotypic variation, and was placed near the marker loci *Xsbarslbk6.03* at position 10.0 cM on LG Sbi06a. It was detected at Havelock in 2009, and the 09178(1) allele decreased moisture content by 2.1% (Table 3).

Conclusions

Genome mapping in sorghum began more than 15 years ago, and several linkage maps have been published, most of which are based primarily on RFLPs with newer maps including other markers such as SSRs and even DArT. As far as we know, this genetic linkage map of sorghum is the first made of SSR markers only. These SSRs produced a map that is in agreement with maps already published, and the total map length was within the estimated map length of sorghum. The progeny phenotypes exceeded the parental range for all the traits measured, which suggest transgressive segregation. Therefore, it was expected to see QTL alleles enhancing traits being inherited from both parents. Since the female parent, 09178(1) is an F_3 nuclear male sterile (ms_3) selection, it might still have been segregating for some traits that led to RILs differences at a particular locus.

In this study, most QTLs for the traits that were significantly correlated are colocalized in a cluster on the distal end of linkage group Sbi01b suggesting the potential importance of chromosome Sbi01 in breeding. The parental lines can be assumed to

adequately represent respective low stem sugar and high stem sugar sorghum types, and these results were comparable to other studies. The mapping of the same QTLs in more than one environment also suggests the stability of the QTL and can be beneficial in sweet sorghum breeding. The colocalized bioenergy QTLs on linkage group Sbi01b, their high positive trait correlation and heritability could provide a platform for future marker assisted selection in sweet sorghum.

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Table 1. Least square means of anthesis date (AD), plant height (PH), moisture content (MC), total biomass yield (BY), and brix at four environments and combined environments in Nebraska.

		Parental lines			RILs			
Trait	Environment	09178(1)	N99	Diff†	Mean	Range	SD‡	H^2 (SE) #
AD	Havelock 2008	90.3	70.8	19.5	73.1	66.9 - 103.2	4.5	0.94 (0.01)
	Havelock 2009	78.5	70.7	7.8	74.0	60.4 - 96.0	6.0	0.93 (0.01)
	Mead 2008	84.3	71.6	12.7	73.7	66.8 - 100.0	4.2	0.98 (0.003)
	Mead 2009	82.1	79.8	2.3	73.0	61.1 - 89.5	5.2	0.90 (0.02)
	Combined	83.8	73.2	10.6	73.4	67.5 90.9	3.0	0.42 (0.07)
PH	Havelock 2008	239.7	223.6	16.1	237.5	147.0 - 317.7	33.2	0.93 (0.01)
	Havelock 2009	246.7	213.9	32.8	222.3	144.4 - 292.2	31.5	0.85 (0.85)
	Mead 2008	239.5	239.1	0.4	226.9	157.8 - 318.8	29.1	0.90 (0.02)
	Mead 2009	287.5	271.2	16.3	254.2	166.3 - 315.7	31.7	0.60 (0.06)
	Combined	253.4	236.9	16.5	235.2	181.6 - 287.4	18.7	0.40 (0.08)
MC	Havelock 2008	64.9	63.1	1.8	62.5	53.4 - 73.3	2.6	0.53 (0.08)
	Havelock 2009	64.9	62.6	2.3	63.9	52.4 - 69.7	3.0	0.78 (0.04)
	Mead 2008	64.0	62.0	2.0	60.7	48.7 - 67.8	3.1	0.83 (0.03)
	Mead 2009	64.1	68.6	-4.5	65.5	41.5 - 75.6	3.3	0.37 (0.10)
	Combined	64.4	64.1	0.3	63.1	56.1 - 67.3	1.8	0.44 (0.07)
BY	Havelock 2008	14.70	10.88	3.8	12.79	6.73 - 24.47	2.78	0.71 (0.05)
	Havelock 2009	17.00	13.73	3.3	15.46	8.46 - 27.33	3.05	0.58 (0.07)
	Mead 2008	16.27	11.26	5.0	11.88	6.06 - 19.27	2.52	0.71 (0.05)
	Mead 2009	17.92	15.18	2.7	14.99	5.39 - 27.78	4.24	0.74 (0.04)
	Combined	16.47	12.76	3.7	13.78	9.45 - 19.11	1.70	0.16 (0.11)
Brix	Havelock 2008	16.5	17.0	-0.5	15.0	8.9 - 20.7	2.2	0.84 (0.03)
	Havelock 2009	14.8	14.4	0.4	15.0	10.0 - 20.1	2.2	0.71 (0.05)
	Mead 2008	17.4	17.8	-0.4	16.8	9.6 - 21.2	2.0	0.80 (0.03)
	Mead 2009	15.0	14.0	1.0	15.0	6.6 - 23.7	2.2	0.67 (0.05)
	Combined	15.9	15.8	0.1	15.5	12.3 - 18.4	1.2	0.32 (0.09)

† - difference between parental lines means. The negative sign indicates that N99 had a great mean.
‡ - standard deviation of the mean.
# - SE is standard error of heritability (in brackets) calculated at $\alpha = 0.05$.

Table 2. Correlation coefficients based on least square means between anthesis date (AD), plant height (PH), moisture content (MC), total biomass yield (BY), and brix of sweet sorghum RILs for the combined environments (n=165).

	AD	PH	MC	BY	Brix
AD	1				
PH	0.17	1			
MC	0.34***	0.18**	1		
BY	0.31***	0.64***	0.04	1	
Brix	-0.05	0.13*	0.07	0.19**	1

*, **, ***, – indicate significance at probability level of 10%, 5%, and 1% respectively; ns indicates non significance at P<0.05.

Table 3. Quantitative trait loci for anthesis date (AD), plant height (PH), moisture content (MC), total biomass yield (BY), and Brix location at Havelock and Mead in 2008 and 2009, and combined location.

QTL	Trait	Linkage group	Environment	LOD score	Flanking markers	Peak Position	Significant marker	R^2 (%)	Additive effect
MC_01	Moisture content	Sbi01b	Havelock 2008	57.18	*Drenhsbm89 - Tx145*	52.6	*Tx145*	77.0	-30.5
MC_02	Moisture content	Sbi06a	Havelock 2009	3.11	*Xcup44 - Xsbarslbk6.32*	10.0	*Xsbarslbk6.03*	7.7	-2.1
PH_01	Plant height	Sbi07	Havelock 2008	4.17	*Xcup68 - SbAGB02*	55.0	*Xsbarslbk7.59*	14.7	-16.7
AD_01	Anthesis date	Sbi01b	Mead 2008	26.80	*Xsbarslbk1.40 - Drenhsbm89*	27.0	*Drenhsbm63*	58.4	-29.02
AD_02	Anthesis date	Sbi01b	Havelock 2009	6.91	*Drenhsbm13 - Xsbarslbk1.56*	167.4	*Xtxtp284*	26.2	-19.1
BY_01	Total biomass yield	Sbi09b	Mead 2008	4.17	*Xabarslbk9.55 - Sam38725*	26.2	*Sam38725*	17.4	-1.23
BY_02	Total biomass yield	Sbi01b	Havelock 2008	4.94	*Xsbarslbk1.56 -Xsbarslbk1.65*	203.4	*Xsbarslbk1.61*	13.5	1.16
BY_02	Total biomass yield	Sbi01b	Mead 2008	4.95	*Xsbarslbk1.56 -Xsbarslbk1.65*	207.9	*Xsbarslbk1.61*	13.2	1.1
BY_03	Total biomass yield	Sbi10b	Havelock 2009	4.05	*Xgap1 - Xsbarslbk10.59*	18.3	*Xcup16*	9.7	-1.19

Table 3. Cont'd

Brix_01	Brix degree	Sbi01b	Havelock 2008	6.06	*Drenhsbm89 - Xcup32*	56.7	*Tx145*	33.9	-6.7
Brix_02	Brix degree	Sbi04b	Combined	3.28	*LBK52 - Xcup28*	39.6	*Xtxtp343*	8.4	-0.6
Brix_02	Brix degree	Sbi04b	Mead 2008	5.83	*LBK48 - Xtxtp343*	26.2	*LBK52*	13.1	-1
Brix_03	Brix degree	Sbi04b	Havelock 2008	3.50	*Drenhsbm27 - Xtxtp158*	52.7	*Xcup28*	7.8	-1
Brix_04	Brix degree	Sbi07	Havelock 2009	3.57	*SbAGB02 - Xcup57*	87.1	*Xsbarslbk7.63*	8.4	-0.8
Brix_05	Brix degree	Sbi01b	Mead 2008	2.90	*Xtxtp284 - Xsbarslbk1.61*	189.4	*Tx147*	6.4	0.7
Brix_06	Brix degree	Sbi05	Mead 2008	2.80	*Xsbarslbk5.05 - Stgnhsbm48*	94.2	*Xsbarslbk5.08*	6.6	-0.7

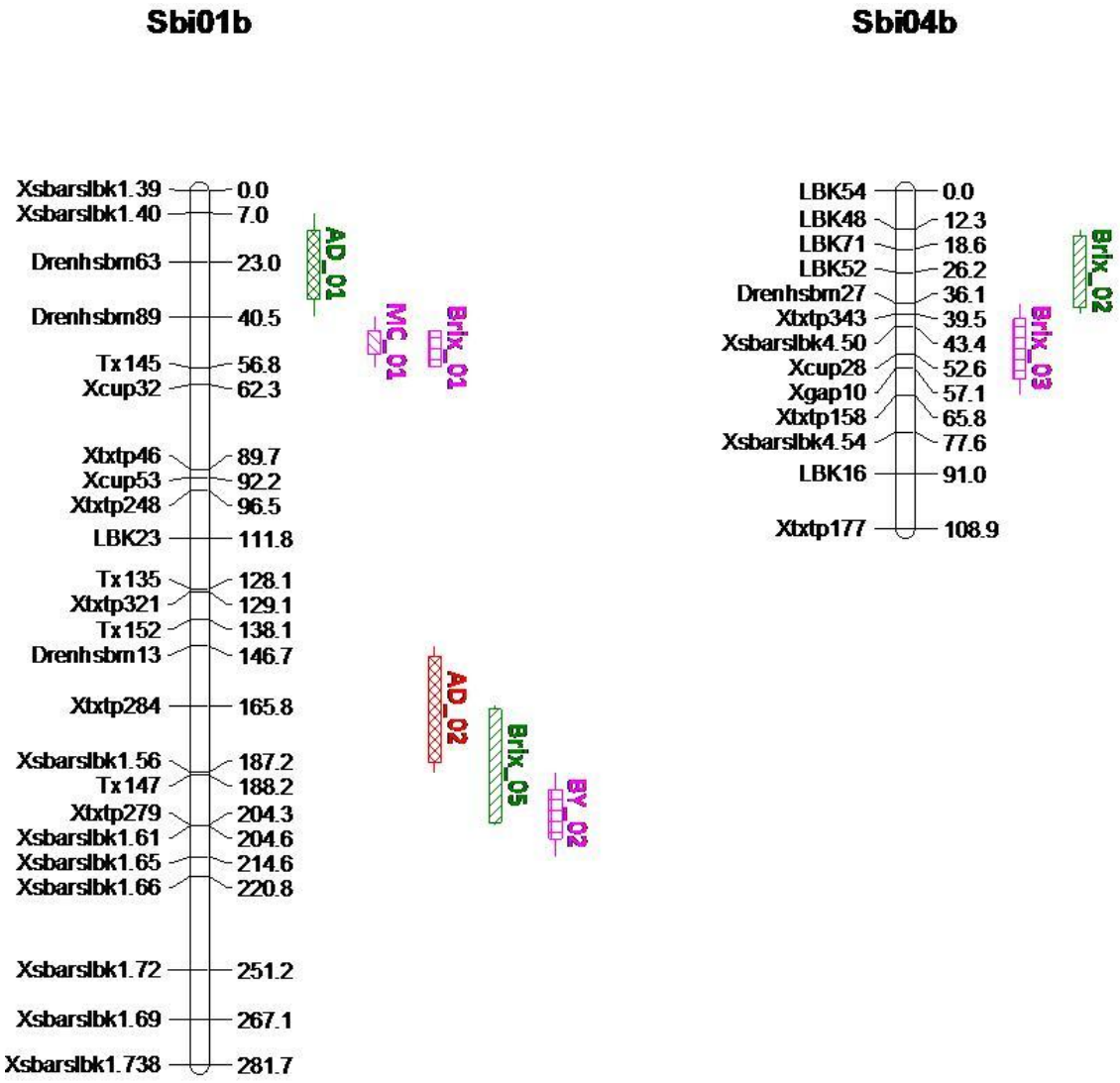


Figure 1. QTL positions on six linkage groups in the sorghum RIL (09178(1) x N99 cross) mapping population evaluated at four environments in Eastern Nebraska. The marker loci names are shown on the left of the linkage group while their positions (cM) are shown on the right side. The bars on the right indicate the regions of the QTL in the linkage group with different shades for different environments. QTLs are color coded as per environment, with purple being Havelock 2008; red is Havelock 2009; and green is Mead 2008. The boxes are also shaded differently for each individual trait.

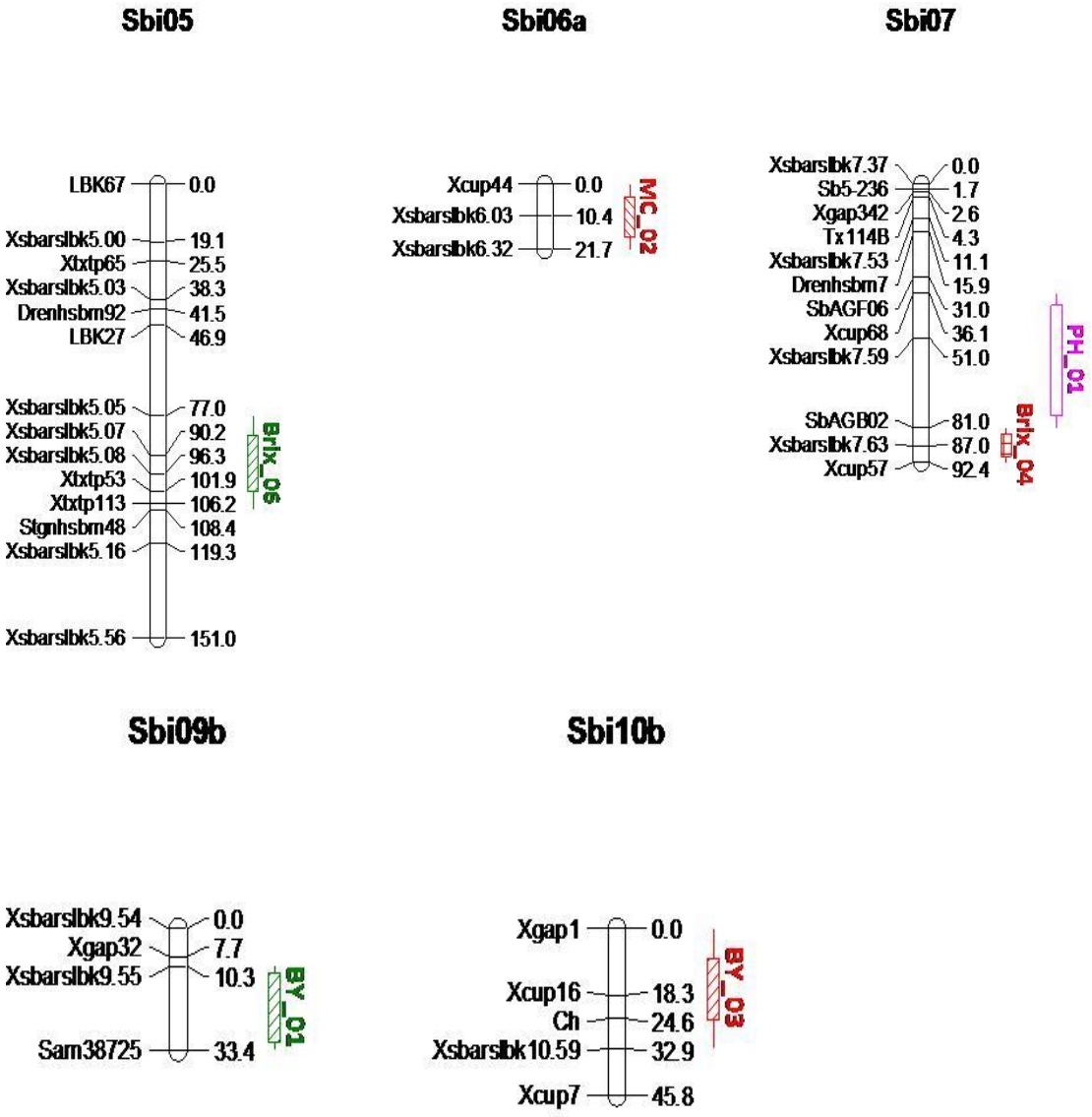
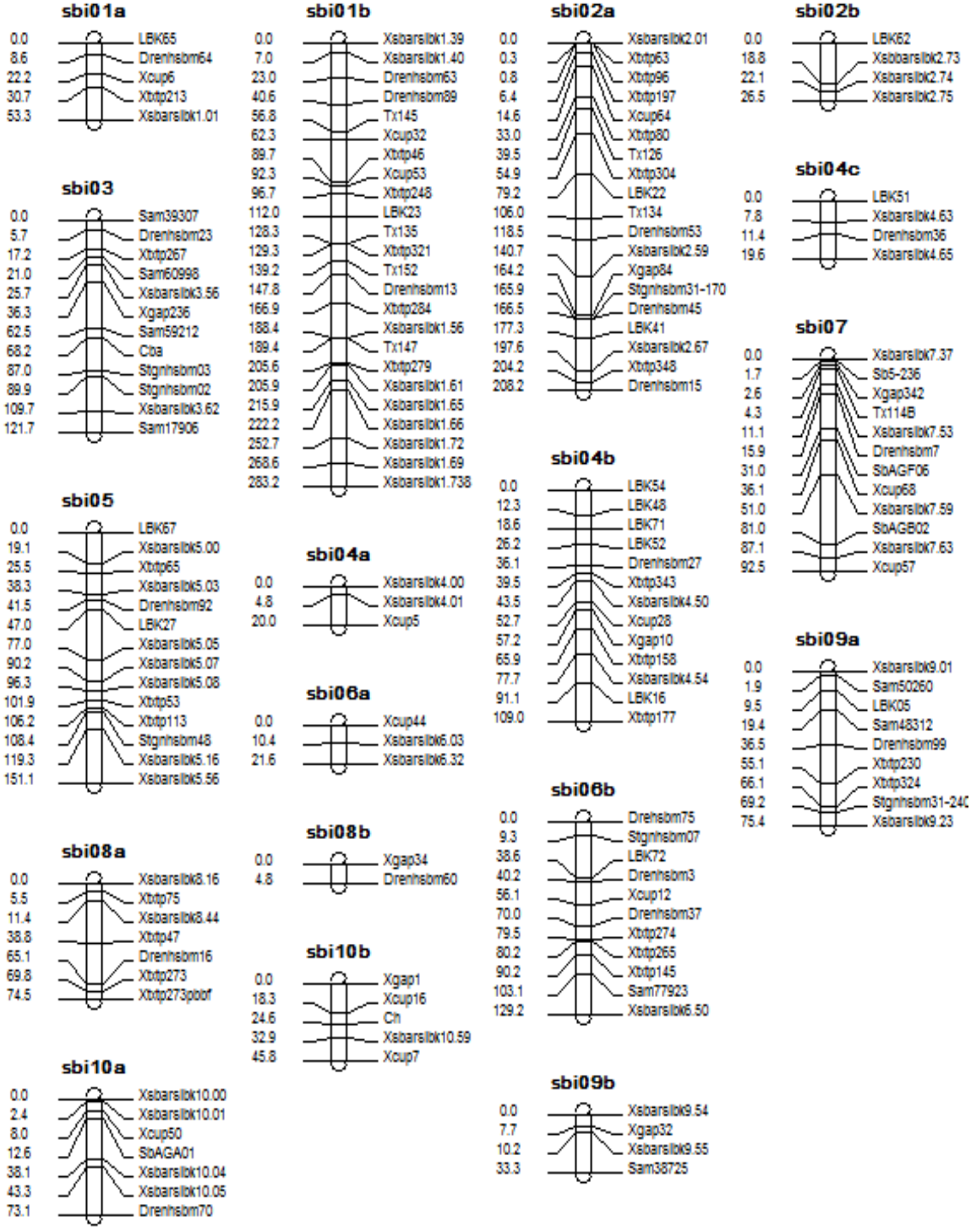


Figure 1. Cont'd

Appendices

Appendix 1. The genetic linkage map of sorghum using 158 SSRs.



Appendix 2. Analysis of variance for anthesis dates, plant height, moisture content, total biomass, and brix for combined environments.

Combined			**Anthesis date**		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Env	3	169.98824	56.662748	5.65	0.0022
Rep(Env)	4	259.44225	64.860562	6.42	0.0003
Block(Env*Rep)	96	713.85985	7.43604	1.61	0.0006
Line	165	11249	68.175282	14.73	<.0001
Env*Line	493	19078	38.697069	8.36	<.0001
Residual	564	2610.3925	4.628356	.	.

			Plant height		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Env	3	198236	66079	118.18	<0.0001
Rep(Env)	4	21925	5481.2	9.75	<0.0001
Block(Env*Rep)	96	44427	462.8	1.29	0.042
Line	165	390640	2367.5	6.61	<0.0001
Env*Line	493	738919	1498.8	4.18	<0.0001
Residual	564	2022007	358.2	.	.

			Moisture content		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Env	3	4379.7	1459.9	60.86	<0.0001
Rep(Env)	4	947.4	236.8	60.31	<0.0001
Block(Env*Rep)	96	1534.8	16	2.38	<0.0001
Line	165	3698.9	22.4	3.34	<0.0001
Env*Line	493	6783.4	13.8	2.05	<0.0001
Residual	563	3776.5	6.7	.	.

			Total biomass		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Env	3	2774.8	924.92	38.25	<0.0001
Rep(Env)	4	387.8	96.96	3.96	0.006
Block(Env*Rep)	96	1490.8	15.53	2.53	<0.0001
Line	165	3374.7	20.45	3.34	<0.0001
Env*Line	493	8904.3	18.06	2.95	<0.0001
Residual	563	3450.3	6.13	.	.

			Brix		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Env	3	731.3	243.76	28.37	<0.0001
Rep(Env)	4	738.2	184.54	21.22	<0.0001
Block(Env*Rep)	96	534.4	5.57	2.45	<0.0001
Line	165	1740	10.55	4.65	<0.0001
Env*Line	493	3584	7.28	3.21	<0.0001
Residual	558	1266.4	2.27	.	.

Appendix 3. Analysis of variance for anthesis date, plant height, moisture content, total biomass, and brix at Havelock for the 2008 Season.

Havelock08			**Anthesis date**		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	5.75	5.75	1.05	0.335
Block(Rep)	24	116.56	4.86	1.15	0.3
Line	165	6790.07	41.15	9.75	<0.0001
Residual	137	578.48	4.22	.	.

			Plant height		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	973.67	973.67	4.69	0.06
Block(Rep)	24	4482.64	186.78	1.14	0.315
Line	165	329258	1995.51	12.13	<0.0001
Residual	137	22542	164.54	.	.

			Moisture content		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	329.42	329.42	12.44	0.003
Block(Rep)	24	398.3	16.6	2.73	0.0001
Line	165	1958.52	11.87	1.96	<0.0001
Residual	137	831.34	6.07	.	.

			Total biomass		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	61.15	61.15	4.72	0.048
Block(Rep)	24	212.58	8.86	1.97	0.008
Line	165	2372.58	14.38	3.2	<0.0001
Residual	137	615.39	4.49	.	.

			Brix		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	273.89	273.89	16.99	0.0005
Block(Rep)	24	217.99	9.08	6.18	<0.0001
Line	165	1461.92	8.91	6.06	<0.0001
Residual	137	194.05	1.47	.	.

Appendix 4. Analysis of variance for anthesis date, plant height, moisture content, total biomass, and brix at Havelock for the 2009 season.

Havelock09			**Anthesis date**		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	195.34	195.34	7.79	0.014
Block(Rep)	24	385.09	16.05	2.42	0.0007
Line	166	10422	62.78	9.49	<0.0001
Residual	144	953.17	6.62	.	.

			Plant height		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	16383	16383	29.07	0.0003
Block(Rep)	24	10440	434.99	1.45	0.096
Line	166	283621	1708.56	5.68	<0.0001
Residual	144	43288	300.61	.	.

			Moisture content		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	563.22	563.22	25.93	<0.0001
Block(Rep)	24	317.2	13.22	3.09	<0.0001
Line	166	2528.6	15.23	3.56	<0.0001
Residual	144	611.71	4.28	.	.

			Total biomass		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	204.68	204.68	8.49	0.011
Block(Rep)	24	381.7	15.9	2.18	0.0003
Line	166	2780.35	16.75	2.3	<0.0001
Residual	144	1042.27	7.29	.	.

			Brix		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	213.83	213.83	18.85	0.0005
Block(Rep)	24	172.09	7.17	2.55	0.0003
Line	166	1417.96	8.54	3.04	<0.0001
Residual	144	404.23	2.81	.	.

Appendix 5. Analysis of variance for anthesis date, plant height, moisture content, total biomass, and brix at Mead for the 2008 season.

Mead08			**Anthesis date**		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	6.01	6.01	2.94	0.117
Block(Rep)	24	38.3	1.6	1.42	0.108
Line	164	5286.49	32.23	28.73	<0.0001
Residual	136	152.59	1.21	.	.

			Plant height		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	4618.73	4618.73	17.1	0.002
Block(Rep)	24	5315.62	221.48	1.3	0.175
Line	164	242530	1478.84	8.68	<0.0001
Residual	136	23161	170.3	.	.

			Moisture content		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	41.42	41.42	1.91	0.184
Block(Rep)	24	310.29	12.93	3.47	<0.0001
Line	164	2858.78	17.43	4.68	<0.0001
Residual	136	506.09	3.72	.	.

			Total biomass		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	93.43	93.43	3.49	0.078
Block(Rep)	24	371.71	15.49	4.31	<0.0001
Line	164	1869.86	11.4	3.18	<0.0001
Residual	136	488.19	3.58	.	.

			Brix		
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	29.43	29.43	9.81	0.01
Block(Rep)	24	55.46	2.31	1.46	0.09
Line	164	1085.5	6.62	4.17	<0.0001
Residual	136	214.31	1.59	.	.

Appendix 6. Analysis of variance for anthesis date, plant height, Moisture content, total biomass, and brix at Mead for the 2009 Season.

Mead09		**Anthesis date**			
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	56.65	56.65	6.62	0.03
Block(Rep)	24	179.28	7.47	1.18	0.27
Line	165	8400.28	50.91	8.03	<0.0001
Residual	145	919.59	6.34	.	.

		Plant height			
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	239.61	239.61	0.19	0.67
Block(Rep)	24	24501	1020.87	1.31	0.16
Line	165	307664	1864.63	2.4	<0.0001
Residual	145	112632	776.77	.	.

		Moisture content			
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	7.42	7.42	0.25	0.63
Block(Rep)	24	510.03	21.25	1.69	0.03
Line	165	3504.19	21.24	1.69	0.0007
Residual	145	1825.06	12.59	.	.

		Total biomass			
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	28.36	28.36	0.89	0.36
Block(Rep)	24	502.09	20.92	2.23	0.002
Line	165	5741.55	34.8	3.71	<0.0001
Residual	145	1361.79	9.39	.	.

		Brix			
Source	**DF**	**SS**	**MS**	**F value**	**P value**
Rep	1	221.49	221.49	51.54	<0.0001
Block(Rep)	24	88.9	3.7	1.2	0.25
Line	165	1384.7	8.39	2.72	<0.0001
Residual	145	447.7	3.08	.	.

Appendix 7. Correlation coefficients between anthesis date, plant height, moisture content, total biomass, and brix at Havelock and Mead, Nebraska in 2008 and 2009 seasons.

Havelock 2008

	AD	PH	MC	BY	Brix
AD	1				
PH	0.20**	1			
MC	0.32***	0.17**	1		
BY	0.37***	0.64***	0.16**	1	
Brix	-0.18**	-0.01ns	0.09ns	-0.05ns	1

Havelock 2009

	AD	PH	MC	BY	Brix
AD	1				
PH	0.05544	1			
MC	0.49***	0.28***	1		
BY	0.17**	0.63***	0.13*	1	
Brix	-0.09ns	0.01ns	0.02ns	0.13ns	1

Mead 2008

	AD	PH	MC	BY	Brix
AD	1				
PH	0.15*	1			
MC	0.27***	0.33***	1		
BY	0.45***	0.52***	0.23***	1	
Brix	0.03ns	0.0001ns	0.04ns	0.16**	1

Mead 2009

	AD	PH	MC	BY	Brix
AD	1				
PH	0.14*	1			
MC	-0.17**	-0.18**	1		
BY	0.26***	0.65***	-0.28***	1	
Brix	0.03ns	0.32***	-0.15*	0.48***	1

*, **, *** indicate significance at P<0.1, 0.05, and 0.01 respectively; ns indicates non significance at P<0.05.

Appendix 8. Least square means for plant stand, anthesis date, plant height, wet weight, moisture content, total biomass, and brix for the combined environments over two seasons (2008 and 2009).

	Combined Environments						
Line	**PS†**	**AD**	**PH‡**	**WWT€**	**MC¥**	**BY¢**	**Brix**
08GHC4-1	22.83	90.88	227.01	44.48	66.32	15.05	16.39
08GHC4-2	25.32	84.57	265.84	51.59	64.48	18.16	17.15
08GHC4-5	22.71	72.68	246.49	43.42	66.80	13.75	17.08
08GHC4-6	23.50	71.32	251.05	47.20	63.96	16.68	17.21
08GHC4-7	22.40	72.66	237.98	32.56	64.97	11.45	16.07
08GHC4-8	22.24	68.22	219.93	30.95	61.09	11.77	16.51
08GHC4-9	23.09	72.38	248.11	40.12	63.29	14.59	16.84
08GHC4-10	20.62	68.69	219.18	31.71	61.15	12.25	15.27
08GHC4-11	21.48	72.26	250.55	40.65	64.03	14.56	13.64
08GHC4-12	19.31	74.59	220.03	34.35	64.21	11.89	16.50
08GHC4-13	24.18	72.48	243.25	36.94	58.96	14.88	15.17
08GHC4-14	22.98	70.96	221.27	36.15	64.04	12.69	16.72
08GHC4-15	23.64	74.90	232.01	34.11	65.09	12.08	16.56
08GHC4-16	22.34	71.32	255.72	39.86	63.24	14.44	16.24
08GHC4-17	22.87	72.86	219.51	34.47	62.46	12.89	14.78
08GHC4-19	20.54	73.09	221.43	31.44	59.23	11.51	13.80
08GHC4-20	22.59	71.46	215.43	37.55	57.61	15.72	17.23
08GHC4-21	24.16	73.00	208.23	36.66	63.19	13.37	18.15
08GHC4-22	23.55	74.94	251.18	41.43	63.53	15.19	13.03
08GHC4-23	22.08	73.54	243.71	42.11	66.58	13.86	15.43
08GHC4-24	24.20	75.79	209.76	29.01	63.91	10.43	14.22
08GHC4-26	25.62	73.21	251.15	36.20	62.84	13.21	15.42
08GHC4-27	24.52	80.34	223.65	30.46	66.15	10.40	14.26
08GHC4-28	22.04	73.08	214.95	39.03	63.92	13.67	14.44
08GHC4-30	22.35	69.98	251.55	36.57	60.92	13.64	15.31
08GHC4-31	23.78	71.35	213.99	37.39	63.06	13.49	15.91
08GHC4-32	19.27	70.75	254.99	45.59	64.25	16.15	14.82
08GHC4-33	22.90	77.24	236.25	37.44	62.44	13.68	15.72
08GHC4-34	23.84	75.32	246.09	34.11	62.70	12.61	14.85
08GHC4-35	25.53	72.51	237.41	41.22	62.94	14.64	14.83
08GHC4-36	21.48	72.17	237.91	40.38	65.32	13.95	14.20
08GHC4-37	23.20	71.37	241.77	32.33	60.35	12.48	14.15
08GHC4-38	23.28	75.60	251.69	43.43	63.71	15.73	14.20
08GHC4-39	22.18	74.90	266.48	41.95	63.96	15.21	16.46
08GHC4-40	21.54	71.75	223.70	41.30	62.26	15.43	17.39
08GHC4-41	20.60	73.24	242.53	36.13	63.28	13.02	15.39
08GHC4-42	23.88	73.45	246.52	38.88	61.92	14.63	14.32
08GHC4-44	23.56	71.33	209.54	33.01	63.47	11.99	15.20
08GHC4-45	24.73	76.35	225.60	35.73	62.83	13.29	16.33
08GHC4-46	22.94	70.94	221.33	33.52	63.41	12.28	18.35
08GHC4-47	22.88	75.35	198.32	38.39	62.58	13.66	14.98
08GHC4-48	23.13	69.94	239.71	42.29	62.47	15.56	15.59
08GHC4-49	22.10	76.83	197.52	36.07	63.96	12.78	13.25

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08GHC4-50	21.96	72.35	218.04	35.33	63.52	12.92	17.24
08GHC4-51	15.65	74.64	190.81	34.80	62.14	13.09	14.39
08GHC4-52	23.27	71.45	225.74	34.96	62.35	13.09	16.26
08GHC4-53	22.32	75.66	238.85	36.00	61.63	13.57	14.39
08GHC4-54	21.03	77.00	232.79	33.22	65.54	11.56	15.54
08GHC4-55	21.69	75.85	204.29	33.85	61.18	12.98	15.10
08GHC4-56	23.10	71.83	251.26	39.77	64.25	14.16	15.55
08GHC4-57	23.32	77.64	246.61	44.24	64.37	15.43	15.20
08GHC4-59	24.02	70.74	242.01	33.76	58.67	12.97	15.37
08GHC4-62	23.63	71.69	230.89	40.81	65.99	13.99	17.72
08GHC4-63	22.06	74.44	241.73	33.54	65.17	11.62	14.00
08GHC4-64	22.02	69.18	251.34	31.85	61.96	12.00	16.95
08GHC4-65	23.74	74.23	233.61	45.05	62.22	16.74	16.21
08GHC4-66	22.39	75.01	252.45	43.98	64.86	15.32	15.35
08GHC4-67	24.79	74.04	232.88	38.57	65.79	13.11	15.55
08GHC4-68	22.95	73.37	258.99	41.75	63.37	15.09	16.46
08GHC4-69	20.11	71.56	253.37	42.29	63.74	15.17	16.08
08GHC4-70	22.90	74.88	235.64	36.58	62.76	13.21	15.32
08GHC4-71	23.33	74.13	244.22	37.61	63.13	13.75	15.78
08GHC4-72	23.27	73.36	235.72	41.41	64.08	14.71	14.30
08GHC4-73	23.14	73.55	257.57	39.34	62.50	14.60	13.20
08GHC4-76	21.72	71.21	207.48	31.66	61.27	11.96	14.79
08GHC4-77	23.39	73.59	241.00	38.18	61.83	14.46	15.02
08GHC4-78	21.63	72.39	241.89	42.69	61.77	16.18	14.88
08GHC4-79	21.49	75.02	219.31	37.99	65.90	12.97	14.17
08GHC4-80	24.52	72.74	204.47	33.65	62.33	12.23	14.47
08GHC4-81	24.13	72.49	212.75	31.96	62.47	11.61	16.20
08GHC4-82	22.23	77.34	240.62	40.13	64.77	14.01	17.03
08GHC4-83	23.66	73.87	260.52	39.35	63.43	14.31	15.26
08GHC4-84	21.59	76.41	230.78	34.89	64.51	12.44	15.43
08GHC4-86	23.97	71.93	220.49	33.90	62.12	12.69	14.72
08GHC4-87	24.22	77.73	235.37	39.60	62.42	14.82	17.48
08GHC4-88	22.40	70.53	212.41	33.93	60.49	13.32	16.94
08GHC4-89	23.04	72.84	219.86	41.74	65.12	13.62	14.98
08GHC4-90	22.28	73.03	203.40	29.13	61.38	11.20	14.79
08GHC4-91	23.24	77.07	225.60	43.61	62.44	16.28	15.38
08GHC4-92	21.96	74.71	238.73	42.10	64.84	14.62	16.09
08GHC4-93	24.33	72.40	235.51	37.52	58.50	15.04	15.27
08GHC4-95	24.48	71.71	221.67	37.51	67.33	12.37	16.72
08GHC4-96	23.26	75.54	253.60	38.07	62.29	14.06	13.14
08GHC4-97	21.25	72.59	216.02	37.36	65.09	13.00	14.42
08GHC4-99	21.94	74.52	210.34	31.31	62.02	11.56	16.27
08GHC4-100	23.48	74.97	234.29	39.58	64.89	13.93	14.11

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08GHC4-102	19.09	72.45	242.09	38.71	63.91	13.88	17.10
08GHC4-103	23.28	77.74	234.38	35.53	60.86	13.76	14.50
08GHC4-104	22.85	71.80	237.29	40.94	64.05	14.35	15.95
08GHC4-105	23.30	72.24	237.28	35.52	61.30	13.58	15.06
08GHC4-106	23.73	71.70	228.74	35.47	62.29	13.27	15.66
08GHC4-108	21.98	73.88	266.81	43.05	60.91	16.56	15.67
08GHC4-109	24.00	69.42	206.43	33.50	62.86	12.05	12.85
08GHC4-110	22.88	77.50	274.61	43.86	64.56	15.33	15.17
08GHC4-112	25.11	76.08	256.88	38.13	64.09	13.67	16.62
08GHC4-113	23.97	72.99	229.93	36.05	61.46	13.71	14.38
08GHC4-114	22.93	69.97	234.63	33.15	56.08	14.01	13.02
08GHC4-116	22.77	76.40	273.64	43.81	61.95	16.64	15.79
08GHC4-117	21.96	71.58	243.85	33.52	60.25	12.99	14.59
08GHC4-118	21.93	74.05	246.14	42.44	64.19	15.09	15.82
08GHC4-125	22.84	77.75	218.83	36.33	63.87	13.10	15.12
08GHC4-126	24.22	76.82	264.76	46.14	63.27	16.58	15.26
08GHC4-127	25.54	67.66	221.86	35.40	59.43	13.72	16.84
08GHC4-128	23.05	71.95	238.91	30.40	63.12	11.24	15.37
08GHC4-129	23.26	71.15	243.42	38.88	64.40	13.74	15.09
08GHC4-130	22.70	72.97	241.16	43.43	63.48	15.76	16.04
08GHC4-131	23.06	76.25	231.47	38.75	66.73	13.03	13.85
08GHC4-132	22.66	74.76	248.08	38.68	64.19	13.75	16.78
08GHC4-133	22.75	69.84	238.62	37.93	64.03	13.63	16.84
08GHC4-134	24.86	71.90	247.14	44.13	62.85	16.20	17.38
08GHC4-135	19.01	71.30	243.65	44.84	66.12	15.15	14.11
08GHC4-136	21.25	70.41	248.92	30.77	63.08	11.49	15.51
08GHC4-137	24.67	72.27	224.33	35.46	61.64	13.21	16.83
08GHC4-140	22.72	71.06	241.97	41.40	62.31	15.43	13.81
08GHC4-141	23.12	76.82	236.07	40.73	64.96	13.96	18.06
08GHC4-142	21.22	72.91	243.99	39.49	57.87	15.77	17.27
08GHC4-143	20.94	75.71	262.73	51.21	64.34	18.09	15.97
08GHC4-145	21.14	73.67	206.67	40.63	64.29	14.62	14.21
08GHC4-147	23.93	71.55	253.60	39.28	63.63	14.34	14.23
08GHC4-148	23.12	75.91	255.18	44.91	63.40	15.95	14.38
08GHC4-149	23.13	75.84	204.35	31.03	62.10	11.42	12.82
08GHC4-150	25.26	71.31	215.25	36.17	62.79	13.43	15.50
08GHC4-151	24.91	74.28	224.69	39.70	62.30	14.57	15.71
08GHC4-152	23.29	70.42	232.44	38.28	63.38	13.76	14.46
08GHC4-153	23.73	74.02	252.49	41.92	64.32	14.86	15.28
08GHC4-154	19.13	76.86	245.10	43.38	64.69	15.09	14.64
08GHC4-157	21.70	70.30	254.65	39.28	63.43	13.92	16.58
08GHC4-159	22.29	73.67	240.83	38.15	61.24	14.43	15.88
08GHC4-160	21.99	68.50	206.08	28.06	62.70	10.19	15.73
08GHC4-161	23.19	74.46	249.24	36.99	63.27	13.50	15.04

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08GHC4-163	22.54	70.55	256.98	38.92	62.28	14.95	14.30
08GHC4-164	22.91	80.53	220.99	43.51	65.90	14.90	14.17
08GHC4-165	22.82	74.17	219.60	38.42	64.81	13.35	16.41
08GHC4-166	21.07	71.63	194.53	29.43	59.32	11.45	15.03
08GHC4-167	20.17	76.17	277.38	50.80	64.39	17.88	16.75
08GHC4-169	24.90	74.62	252.63	42.54	60.11	16.56	15.95
08GHC4-170	22.15	75.29	248.77	38.02	64.61	13.36	16.16
08GHC4-171	21.99	74.58	252.95	45.66	63.16	16.64	15.72
08GHC4-172	20.07	74.11	244.47	39.23	63.26	14.44	16.99
08GHC4-173	18.62	73.97	255.79	40.57	64.11	14.17	14.24
08GHC4-174	22.58	77.52	232.68	42.57	63.09	15.37	13.84
08GHC4-176	24.44	72.64	237.77	34.14	63.91	12.42	14.93
08GHC4-180	24.29	74.34	248.82	43.14	63.48	15.71	15.73
08GHC4-181	23.77	67.53	222.21	34.96	62.43	12.75	17.36
08GHC4-183	22.08	69.39	219.48	32.07	62.69	11.89	14.65
08GHC4-184	20.64	73.06	254.52	43.17	63.07	15.78	15.83
08GHC4-185	22.15	70.30	218.09	27.66	62.79	9.87	15.30
08GHC4-186	25.07	72.64	223.57	30.35	62.29	11.25	18.35
08GHC4-187	22.78	70.60	195.98	35.13	62.10	12.90	16.34
08GHC4-188	26.06	71.62	247.45	43.19	65.55	14.75	14.84
08GHC4-190	24.44	71.41	181.63	27.90	61.49	10.51	12.29
08GHC4-191	25.04	71.68	219.42	37.16	64.33	12.42	17.28
08GHC4-192	23.61	75.08	245.84	40.90	65.68	13.92	13.84
08GHC4-193	25.24	70.51	242.34	37.86	63.71	13.58	16.11
08GHC4-194(1)	20.41	72.08	250.49	38.35	63.09	14.07	17.05
08GHC4-194(2)	22.13	73.33	236.36	33.77	63.45	12.32	13.63
08GHC4-195	26.72	69.94	238.16	35.18	63.44	12.74	15.42
08GHC4-197	24.96	70.93	232.48	26.28	61.87	9.45	12.91
08GHC4-198	19.92	72.81	232.41	32.98	64.58	11.56	15.01
08GHC4-200	19.83	75.83	287.41	52.05	63.20	19.11	17.31
08GHC4-201	.	.	.	.	.	.	.
08GHC4-202	20.17	75.60	235.07	37.06	61.62	14.15	14.04
08GHC4-203	22.05	73.04	259.44	38.11	63.49	13.82	16.05
08GHC4-204	22.50	72.21	189.07	27.34	60.09	10.72	15.07
09178(1)(P1)	21.47	83.76	253.36	46.13	64.44	16.47	15.93
N99(P2)	23.51	73.22	236.94	36.26	64.10	12.76	15.82
Mean	**22.75**	**73.50**	**235.27**	**37.99**	**63.10**	**13.79**	**15.46**
SE	**1.14**	**0.79**	**6.83**	**2.58**	**0.96**	**0.92**	**0.56**
Line Mean	**22.76**	**73.44**	**235.15**	**37.95**	**63.08**	**13.78**	**15.45**

† - plant stand; AD – anthesis date; ‡ - plant height; € - wet weight; ¥ - moisture content; £ - total biomass

Appendix 9. Least square means for plant stand, anthesis date, plant height, wet weight, moisture contenttotal biomass, and brix at Havelock, Lincoln, Nebraska for 2008 season.

Havelock 2008							
Line	**PS**	**AD**	**PH**	**WWT**	**MC**	**BY**	**Brix**
08GHC4-1	19.40	103.20	226.40	40.81	67.20	13.39	14.74
08GHC4-2	22.26	97.07	249.22	46.96	64.07	16.69	14.58
08GHC4-5	19.04	67.82	237.09	32.62	63.31	11.94	17.86
08GHC4-6	19.87	68.34	238.41	38.29	64.74	13.46	18.83
08GHC4-7	22.61	69.70	248.04	35.33	64.87	12.45	18.65
08GHC4-8	17.95	70.06	231.38	23.23	60.62	9.26	17.87
08GHC4-9	21.53	71.48	235.42	29.28	65.43	10.22	15.40
08GHC4-10	19.73	66.96	211.75	28.70	62.19	10.51	17.12
08GHC4-11	19.38	70.79	259.04	34.66	63.91	12.34	11.17
08GHC4-12	16.32	69.71	239.29	23.68	64.37	8.41	16.50
08GHC4-13	19.58	74.89	224.53	32.79	59.30	13.26	12.24
08GHC4-14	20.81	66.93	219.84	25.25	63.07	9.41	20.20
08GHC4-15	21.26	70.52	247.85	35.50	73.34	10.32	16.90
08GHC4-16	20.03	71.32	272.11	35.85	61.89	13.50	14.78
08GHC4-17	20.47	73.55	196.47	29.72	63.46	10.97	13.77
08GHC4-19	18.80	68.60	179.07	21.47	55.66	9.01	11.23
08GHC4-20	20.47	71.13	203.07	24.50	55.93	10.98	12.50
08GHC4-21	23.04	73.76	166.62	30.75	63.26	11.29	20.65
08GHC4-22	21.27	76.07	267.20	51.25	62.70	19.13	14.51
08GHC4-23	19.34	74.31	234.37	40.33	64.63	14.36	12.14
08GHC4-24	19.12	74.56	194.26	30.64	66.20	10.35	13.89
08GHC4-26	17.95	76.82	273.06	37.76	62.53	13.96	12.68
08GHC4-27	21.47	76.43	246.28	32.45	65.54	11.19	12.70
08GHC4-28	18.83	70.99	177.50	31.93	63.19	11.69	12.10
08GHC4-30	20.55	72.12	247.90	24.88	57.34	10.76	14.59
08GHC4-31	17.80	70.17	175.65	24.36	63.49	8.71	16.69
08GHC4-32	13.45	75.71	272.21	42.96	66.09	14.55	14.82
08GHC4-33	18.95	73.71	235.86	24.97	60.00	9.97	12.91
08GHC4-34	20.10	76.51	243.18	30.72	65.99	10.53	13.84
08GHC4-35	20.21	69.11	223.81	28.26	60.18	11.28	11.71
08GHC4-36	18.49	68.80	240.20	34.83	64.31	12.49	14.12
08GHC4-37	21.57	69.96	249.92	28.09	57.59	11.69	14.73
08GHC4-38	14.81	73.12	259.78	42.94	62.13	16.19	14.86
08GHC4-39	19.70	75.85	314.58	48.98	61.79	19.00	15.95
08GHC4-40	17.59	72.75	197.65	47.19	61.82	17.86	16.31
08GHC4-41	18.52	73.46	248.08	35.60	60.02	14.20	17.21
08GHC4-42	21.90	77.91	263.45	37.25	61.49	14.42	12.88
08GHC4-44	19.13	75.10	249.63	37.48	63.85	13.54	13.84
08GHC4-45	20.20	73.56	193.32	23.88	63.39	8.88	15.80
08GHC4-46	19.07	72.98	187.14	27.24	65.09	9.55	19.72
08GHC4-47	19.96	76.47	179.24	27.41	62.95	10.01	14.96
08GHC4-48	21.74	70.04	227.28	37.07	57.74	15.69	13.12
08GHC4-49	20.82	74.01	193.27	36.33	66.31	12.23	10.59

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08GHC4-50	21.09	75.81	223.53	30.49	63.58	11.11	16.48
08GHC4-51	4.87	75.79	198.55	26.65	65.88	9.06	13.94
08GHC4-52	20.41	77.65	198.51	30.54	60.23	12.37	14.88
08GHC4-53	17.02	73.49	260.01	33.34	59.44	13.61	13.50
08GHC4-54	19.31	77.04	279.31	42.65	66.40	14.58	12.54
08GHC4-55	14.95	72.41	168.17	22.74	59.14	9.56	.
08GHC4-56	22.37	70.42	244.90	30.68	63.41	11.33	15.00
08GHC4-57	19.24	75.48	259.18	36.48	62.21	13.66	14.88
08GHC4-59	22.31	72.64	241.88	28.13	54.80	12.61	12.94
08GHC4-62	18.77	71.86	226.09	32.93	65.82	11.27	17.03
08GHC4-63	18.67	76.04	290.76	30.17	64.62	10.70	12.06
08GHC4-64	23.87	68.70	254.68	26.06	61.98	9.63	17.37
08GHC4-65	20.89	72.54	234.29	39.06	60.59	15.27	15.99
08GHC4-66	20.34	74.55	251.85	47.94	65.08	16.71	11.74
08GHC4-67	19.81	77.93	231.84	35.12	61.55	13.57	17.22
08GHC4-68	16.37	74.19	262.24	30.93	62.02	11.78	14.13
08GHC4-69	15.75	74.57	270.97	37.58	62.67	14.06	13.63
08GHC4-70	21.46	71.66	206.84	25.94	60.24	10.31	14.94
08GHC4-71	15.45	76.29	244.19	35.75	62.35	13.42	13.05
08GHC4-72	19.16	74.43	247.73	44.27	65.00	15.57	12.76
08GHC4-73	19.17	72.40	233.96	35.25	61.74	13.45	14.56
08GHC4-76	17.99	73.47	186.96	23.31	60.92	9.08	13.11
08GHC4-77	21.56	72.19	247.58	35.10	61.98	13.34	14.36
08GHC4-78	18.06	73.41	235.88	32.38	59.18	13.36	12.85
08GHC4-79	19.27	72.52	236.83	40.84	62.01	15.59	12.74
08GHC4-80	17.72	69.12	197.42	24.67	60.72	9.66	11.93
08GHC4-81	20.42	68.40	199.90	22.53	59.04	9.21	16.82
08GHC4-82	18.54	70.74	247.41	44.06	64.26	15.54	16.83
08GHC4-83	23.41	73.79	275.02	42.66	63.41	15.69	13.98
08GHC4-84	21.10	74.73	256.16	38.64	63.29	14.29	16.64
08GHC4-86	17.98	69.32	218.78	31.69	60.48	12.46	13.66
08GHC4-87	20.33	78.37	264.87	44.56	61.90	16.93	16.92
08GHC4-88	18.29	72.71	226.02	30.75	63.50	11.21	16.89
08GHC4-89	23.18	68.38	181.98	22.63	61.03	8.80	16.13
08GHC4-90	19.66	74.31	213.30	29.38	61.88	11.21	14.74
08GHC4-91	22.37	80.55	216.59	36.35	63.74	13.08	14.21
08GHC4-92	19.16	73.67	240.14	36.66	65.29	12.52	16.46
08GHC4-93	23.38	70.55	220.57	24.99	57.04	10.93	13.66
08GHC4-95	20.95	77.41	238.17	36.20	66.19	12.30	15.51
08GHC4-96	21.43	76.54	258.01	40.52	63.38	14.82	15.04
08GHC4-97	18.71	74.38	273.42	46.78	63.51	17.05	14.03
08GHC4-99	19.95	76.41	214.17	41.20	64.45	14.63	16.39
08GHC4-100	19.00	78.23	233.27	39.34	66.14	13.37	15.55
08GHC4-102	17.45	71.79	235.74	38.27	64.74	13.45	15.35
08GHC4-103	20.75	74.12	254.36	32.56	60.40	12.99	11.80
08GHC4-104	18.23	72.96	261.10	45.81	65.75	15.52	14.63

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08GHC4-105	21.38	73.11	259.25	35.73	62.92	13.25	10.55
08GHC4-106	18.78	76.49	279.06	35.32	60.48	14.07	17.46
08GHC4-108	18.95	71.32	232.76	26.09	61.31	9.87	14.09
08GHC4-109	21.15	69.93	175.55	29.70	60.52	11.71	12.90
08GHC4-110	19.98	75.90	294.35	42.85	61.15	16.73	16.97
08GHC4-112	20.16	75.10	284.35	31.06	62.57	11.63	15.80
08GHC4-113	20.27	73.59	254.74	35.83	62.77	13.34	11.38
08GHC4-114	20.68	67.93	221.27	20.75	53.41	9.91	12.78
08GHC4-116	19.71	75.13	288.00	33.35	60.22	13.49	17.36
08GHC4-117	19.55	72.12	251.97	34.43	61.67	13.24	12.82
08GHC4-118	16.59	74.76	253.48	45.17	62.72	16.55	16.28
08GHC4-125	20.59	74.12	229.36	36.51	62.03	13.91	15.88
08GHC4-126	21.15	73.09	235.30	24.72	62.39	9.10	13.32
08GHC4-127	21.03	67.99	204.99	25.15	58.13	10.78	15.50
08GHC4-128	19.88	68.99	261.59	26.50	63.67	9.74	16.86
08GHC4-129	21.28	68.29	227.75	31.04	64.54	10.94	16.01
08GHC4-130	20.39	71.26	242.93	39.45	61.83	15.04	17.10
08GHC4-131	18.14	75.76	268.20	44.98	64.08	16.00	11.23
08GHC4-132	12.82	71.06	255.64	31.76	65.76	10.99	18.73
08GHC4-133	21.48	69.04	231.21	33.79	62.96	12.55	17.90
08GHC4-134	20.25	69.34	235.01	38.28	64.86	13.63	18.57
08GHC4-135	12.44	73.13	248.52	41.60	66.11	14.06	15.78
08GHC4-136	18.09	71.06	266.68	37.34	62.95	13.87	17.77
08GHC4-137	21.35	66.96	179.63	19.08	59.14	7.60	18.88
08GHC4-140	20.48	69.74	218.99	37.19	62.95	13.65	12.23
08GHC4-141	22.03	73.19	239.86	32.52	62.37	12.19	17.22
08GHC4-142	16.65	70.71	242.84	47.24	65.19	16.66	17.78
08GHC4-143	20.22	78.43	289.73	51.50	63.52	18.70	14.81
08GHC4-145	17.03	76.83	181.24	32.80	66.53	10.99	12.89
08GHC4-147	21.33	71.37	269.69	39.05	63.21	14.46	16.68
08GHC4-148	19.31	70.39	271.13	33.79	63.10	12.38	13.41
08GHC4-149	21.25	74.05	193.87	25.00	62.27	9.46	12.44
08GHC4-150	21.50	76.50	220.61	30.76	65.77	10.66	14.08
08GHC4-151	20.16	76.24	196.48	34.29	60.64	13.39	16.72
08GHC4-152	21.70	72.62	253.92	39.65	61.73	15.11	12.28
08GHC4-153	20.89	73.99	244.58	31.04	62.36	11.81	12.80
08GHC4-154	18.31	70.97	271.94	41.83	61.67	15.95	15.64
08GHC4-157	18.67	72.68	235.07	26.75	60.47	10.35	14.55
08GHC4-159	19.57	72.44	281.89	33.43	62.42	12.66	12.85
08GHC4-160	21.73	68.27	215.74	30.46	61.13	11.79	18.21
08GHC4-161	20.89	75.44	290.22	40.17	64.17	14.62	13.32
08GHC4-163	20.23	71.07	266.05	43.10	60.54	17.10	14.70
08GHC4-164	18.11	82.16	192.36	42.75	67.45	13.95	11.93
08GHC4-165	19.00	70.87	208.31	30.13	62.00	11.32	15.88
08GHC4-166	14.65	69.90	184.80	20.91	56.54	9.07	15.91
08GHC4-167	17.30	78.62	317.67	72.12	65.74	24.47	17.15

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08GHC4-169	21.02	73.39	256.80	33.74	57.82	13.95	18.02
08GHC4-170	18.55	76.14	263.82	34.86	64.37	12.47	15.23
08GHC4-171	17.77	73.99	275.08	54.56	62.17	20.47	13.94
08GHC4-172	17.85	74.73	288.23	36.87	63.79	13.40	17.83
08GHC4-173	11.81	72.54	263.77	29.23	61.42	11.29	12.12
08GHC4-174	18.38	72.65	216.88	33.10	61.16	12.66	14.67
08GHC4-176	22.35	69.69	264.79	36.90	62.47	13.83	13.18
08GHC4-180	21.42	71.56	257.03	45.06	62.85	16.83	14.13
08GHC4-181	19.53	68.23	219.39	29.44	57.82	12.60	16.33
08GHC4-183	17.76	68.96	238.48	28.71	61.12	10.88	14.91
08GHC4-184	15.58	70.99	292.43	48.99	62.19	18.58	15.96
08GHC4-185	13.66	69.48	179.10	16.82	59.57	6.73	15.82
08GHC4-186	20.35	69.16	221.09	27.33	61.19	10.66	19.62
08GHC4-187	20.48	68.58	164.46	25.64	61.72	9.82	17.16
08GHC4-188	22.09	70.87	256.75	39.99	62.75	14.90	17.34
08GHC4-190	22.83	77.55	191.87	38.67	63.55	13.70	8.90
08GHC4-191	20.14	66.91	181.38	21.98	64.64	7.85	18.54
08GHC4-192	20.89	72.31	242.89	43.69	65.42	15.14	12.43
08GHC4-193	19.51	67.62	251.18	24.90	62.99	9.15	18.41
08GHC4-194(1)	14.44	74.08	285.16	42.98	62.68	16.17	16.79
08GHC4-194(2)	19.05	73.80	273.08	37.98	61.91	14.49	16.26
08GHC4-195	20.49	68.74	213.97	27.75	60.71	10.78	15.61
08GHC4-197	22.38	72.03	265.39	27.58	63.66	9.98	11.36
08GHC4-198	20.48	69.17	253.69	33.08	62.76	12.33	14.50
08GHC4-200	20.18	75.33	310.25	49.14	63.45	17.87	17.04
08GHC4-201	.	.	.	.	.	.	.
08GHC4-202	19.33	73.10	254.79	31.12	59.24	12.60	12.52
08GHC4-203	21.56	79.57	272.61	44.90	64.75	16.00	15.06
08GHC4-204	20.43	68.73	147.00	19.71	61.36	7.35	14.06
09178(1)(P1)	20.32	90.28	239.73	42.03	64.89	14.70	16.52
N99(P2)	20.42	70.75	223.60	29.48	63.13	10.88	17.04
Mean	**19.48**	**73.17**	**237.44**	**34.38**	**62.51**	**12.79**	**14.98**
SE	**2.29**	**1.57**	**13.66**	**5.15**	**1.92**	**1.84**	**1.12**
Line Mean	**19.46**	**73.08**	**237.51**	**34.36**	**62.49**	**12.79**	**14.96**

Appendix 10. Least square means for plant stand, anthesis date, plant height, wet weight, moisture content, total biomass, and brix at Havelock, Lincoln, Nebraska for the 2009 season.

Havelock 2009							
Line	**PS**	**AD**	**PH**	**WWT**	**MC**	**BY**	**Brix**
08GHC4-1	25.51	95.97	183.48	49.11	67.69	15.86	17.76
08GHC4-2	24.57	74.68	262.83	65.62	65.31	22.51	19.23
08GHC4-5	24.50	78.10	253.90	55.56	68.97	17.25	15.66
08GHC4-6	26.04	73.60	252.07	68.32	64.47	24.06	14.01
08GHC4-7	22.25	78.54	188.32	30.05	65.97	10.35	14.76
08GHC4-8	26.42	66.65	257.35	45.66	62.51	17.22	20.08
08GHC4-9	21.10	72.10	231.49	45.95	62.91	16.83	16.01
08GHC4-10	18.57	71.40	186.27	32.45	57.51	14.00	13.94
08GHC4-11	18.05	75.23	164.72	46.04	64.61	16.34	17.49
08GHC4-12	23.23	88.49	153.76	41.79	68.09	13.17	15.61
08GHC4-13	22.63	72.65	227.98	40.71	59.70	16.36	14.82
08GHC4-14	27.37	77.24	229.40	39.01	64.25	13.82	15.42
08GHC4-15	22.13	73.39	193.18	32.94	61.65	12.81	14.17
08GHC4-16	22.46	68.75	233.14	44.82	64.95	15.65	17.83
08GHC4-17	24.69	69.71	201.08	29.92	62.28	11.28	14.12
08GHC4-19	19.02	79.49	274.04	45.26	62.69	16.75	16.79
08GHC4-20	22.53	68.36	171.54	31.68	59.93	12.55	15.53
08GHC4-21	22.55	69.60	241.76	42.88	65.63	14.73	16.64
08GHC4-22	18.32	69.62	261.27	48.15	64.50	16.77	15.38
08GHC4-23	22.51	74.00	258.21	51.95	67.66	16.17	16.35
08GHC4-24	25.26	78.73	226.61	32.74	62.94	12.15	10.29
08GHC4-26	33.23	64.89	195.40	28.80	61.01	11.08	16.77
08GHC4-27	25.20	89.89	191.10	32.17	68.97	10.18	16.49
08GHC4-28	19.16	78.65	226.28	41.58	64.22	14.89	12.97
08GHC4-30	22.22	62.81	249.73	41.51	63.41	15.27	15.85
08GHC4-31	26.85	79.22	235.04	64.16	64.70	22.30	14.67
08GHC4-32	26.07	63.16	212.42	45.98	61.64	17.43	12.43
08GHC4-33	23.09	80.93	250.97	47.78	65.74	16.20	18.18
08GHC4-34	25.30	75.22	232.30	41.95	58.09	17.85	14.37
08GHC4-35	27.83	83.40	264.30	61.49	66.58	20.19	15.57
08GHC4-36	24.45	75.97	226.06	47.44	65.83	16.07	13.77
08GHC4-37	25.99	66.32	232.67	48.44	64.50	16.90	12.10
08GHC4-38	29.11	68.74	249.88	34.77	65.02	12.23	11.26
08GHC4-39	20.36	79.15	245.94	36.68	64.79	13.01	16.35
08GHC4-40	25.84	65.54	233.90	37.12	63.39	13.53	17.18
08GHC4-41	22.28	77.56	231.99	45.40	65.44	15.62	13.29
08GHC4-42	26.50	67.29	215.46	32.62	61.66	12.14	13.18
08GHC4-44	28.18	66.53	178.61	40.23	63.04	14.55	14.57
08GHC4-45	27.64	77.67	241.13	45.86	63.84	16.57	16.22
08GHC4-46	26.13	65.72	239.17	44.04	62.94	16.30	16.87
08GHC4-47	23.12	75.18	160.67	32.08	61.21	12.09	13.19
08GHC4-48	24.26	69.24	245.07	47.69	67.63	15.50	15.97

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08GHC4-49	22.34	85.47	185.12	46.73	65.95	15.73	13.94
08GHC4-50	20.73	70.82	187.67	32.84	64.92	11.85	18.38
08GHC4-51	18.89	80.98	144.39	39.73	60.97	15.38	16.63
08GHC4-52	23.25	69.32	245.21	43.16	63.96	15.76	16.59
08GHC4-53	25.57	79.95	224.18	52.22	64.83	18.03	12.91
08GHC4-54	18.92	74.50	155.66	28.04	67.94	9.12	19.79
08GHC4-55	22.37	74.16	193.66	38.14	64.42	13.66	13.19
08GHC4-56	25.44	69.71	242.50	44.15	63.80	15.95	17.93
08GHC4-57	20.79	79.89	239.79	47.37	68.60	14.80	15.50
08GHC4-59	26.55	68.49	240.12	46.56	64.04	16.64	17.95
08GHC4-62	22.87	71.81	266.56	49.73	65.45	17.24	17.85
08GHC4-63	22.17	74.40	209.80	42.95	65.29	14.83	12.47
08GHC4-64	17.27	67.90	235.28	39.35	65.16	13.85	18.60
08GHC4-65	25.63	76.08	232.98	53.25	65.12	18.40	15.68
08GHC4-66	23.46	81.89	238.99	45.62	66.33	15.25	17.43
08GHC4-67	27.71	77.81	208.68	46.44	68.71	14.59	11.83
08GHC4-68	27.79	71.04	242.78	47.00	65.68	16.15	18.41
08GHC4-69	23.11	69.77	239.14	54.73	65.33	19.09	17.68
08GHC4-70	24.53	80.77	254.07	51.34	66.53	17.26	14.70
08GHC4-71	29.73	75.28	243.68	42.61	66.63	14.05	14.45
08GHC4-72	22.09	69.98	175.53	29.51	59.94	11.74	16.44
08GHC4-73	24.90	78.50	279.04	52.16	64.25	18.54	10.04
08GHC4-76	22.92	68.57	184.91	29.90	61.40	11.09	11.94
08GHC4-77	22.91	74.91	261.38	48.95	61.28	18.90	12.75
08GHC4-78	23.30	67.60	244.29	48.58	65.65	16.86	15.38
08GHC4-79	21.96	81.58	228.42	43.78	69.36	13.53	11.11
08GHC4-80	25.93	77.32	237.28	45.81	64.19	16.42	13.07
08GHC4-81	24.35	76.41	258.02	50.90	66.46	16.94	16.20
08GHC4-82	25.06	84.59	215.71	43.16	67.49	13.96	15.69
08GHC4-83	22.33	73.25	238.78	36.44	60.91	14.30	15.80
08GHC4-84	26.94	75.81	192.31	40.55	64.77	14.33	14.92
08GHC4-86	25.39	73.89	241.60	39.32	62.73	14.75	11.00
08GHC4-87	27.72	70.71	232.99	45.60	60.72	17.88	18.54
08GHC4-88	23.04	61.32	160.64	28.22	57.42	11.83	17.39
08GHC4-89	26.27	73.81	234.28	50.21	69.01	15.53	13.51
08GHC4-90	19.47	74.48	211.29	33.43	63.04	12.03	14.29
08GHC4-91	25.84	71.85	226.45	51.24	63.89	18.38	16.02
08GHC4-92	25.63	82.03	241.56	48.41	67.77	15.60	13.71
08GHC4-93	22.52	79.84	232.89	40.23	60.10	15.86	15.14
08GHC4-95	29.67	71.82	246.76	44.30	64.33	15.81	18.96
08GHC4-96	22.33	69.01	230.25	25.21	55.84	11.22	11.13
08GHC4-97	17.91	68.92	149.49	33.84	67.26	11.11	13.90
08GHC4-99	17.74	68.10	163.38	28.90	52.41	13.55	16.64
08GHC4-100	27.57	68.99	215.02	42.05	63.50	15.37	12.22
08GHC4-102	19.40	60.40	244.71	41.63	62.78	15.61	16.96

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08GHC4-103	26.10	72.57	183.40	45.98	62.92	16.75	15.39
08GHC4-104	22.47	70.28	191.16	41.15	61.73	15.71	16.36
08GHC4-105	24.26	70.03	166.69	27.55	60.26	10.72	16.31
08GHC4-106	27.53	71.24	221.70	39.55	61.53	15.49	13.02
08GHC4-108	23.55	73.78	292.23	65.62	61.77	25.16	16.56
08GHC4-109	24.38	72.57	231.21	49.48	64.97	16.96	15.02
08GHC4-110	18.75	78.00	260.19	50.87	69.69	15.35	12.07
08GHC4-112	27.72	75.60	237.85	36.46	67.16	11.94	14.04
08GHC4-113	30.64	70.62	172.76	34.60	59.04	14.09	15.07
08GHC4-114	24.73	69.68	226.02	42.28	59.99	16.54	11.92
08GHC4-116	23.54	78.49	237.73	37.56	63.65	13.63	12.92
08GHC4-117	23.85	69.24	207.49	23.79	55.18	10.43	14.28
08GHC4-118	21.05	76.12	242.80	57.51	65.64	19.63	14.70
08GHC4-125	23.05	80.40	248.55	39.18	67.30	12.85	11.82
08GHC4-126	24.53	83.31	282.75	77.95	64.65	27.33	16.27
08GHC4-127	29.65	62.18	180.94	26.56	57.82	11.25	15.15
08GHC4-128	22.18	74.22	172.35	31.84	63.85	11.61	15.49
08GHC4-129	22.74	76.26	256.68	52.32	64.04	18.63	15.39
08GHC4-130	20.34	75.14	231.37	45.09	67.36	14.75	12.63
08GHC4-131	25.78	80.27	252.49	46.47	63.89	16.80	17.85
08GHC4-132	28.09	75.91	239.00	40.90	63.94	14.63	15.89
08GHC4-133	20.07	65.45	216.06	26.49	68.38	8.46	14.06
08GHC4-134	25.40	70.46	231.18	38.01	61.41	14.72	14.39
08GHC4-135	19.61	75.91	244.99	66.32	66.53	22.20	12.17
08GHC4-136	19.06	73.25	229.87	39.83	61.34	15.41	13.12
08GHC4-137	27.12	81.54	267.69	57.54	65.37	20.21	14.05
08GHC4-140	24.28	75.49	242.20	46.70	63.52	17.14	12.07
08GHC4-141	20.91	90.48	197.33	42.92	68.24	13.57	17.81
08GHC4-142	25.11	67.49	211.53	31.05	64.02	10.53	13.49
08GHC4-143	13.99	76.24	241.16	59.98	66.10	20.09	16.09
08GHC4-145	20.45	66.89	213.02	38.05	63.11	14.00	14.57
08GHC4-147	24.79	74.65	247.23	49.31	61.64	18.89	11.60
08GHC4-148	23.31	78.99	170.64	35.12	62.13	13.16	12.31
08GHC4-149	26.59	73.80	231.86	54.44	65.39	18.72	15.16
08GHC4-150	26.58	63.23	200.52	32.52	58.51	13.41	13.07
08GHC4-151	29.60	77.31	274.59	57.53	64.49	20.19	12.18
08GHC4-152	22.82	66.49	188.83	33.95	62.15	12.41	14.38
08GHC4-153	26.63	69.22	231.71	48.68	65.21	17.22	18.59
08GHC4-154	18.24	85.70	166.50	30.49	64.78	10.61	14.35
08GHC4-157	18.43	69.40	252.47	51.30	65.18	17.66	16.40
08GHC4-159	22.35	68.91	196.97	45.31	63.49	16.28	16.35
08GHC4-160	24.31	67.28	225.26	45.23	65.51	15.41	15.51
08GHC4-161	21.45	68.35	217.33	37.37	61.06	14.37	12.76
08GHC4-163	16.85	73.13	248.79	49.99	62.59	18.81	13.29
08GHC4-164	29.93	76.99	255.46	49.19	65.61	16.81	12.23

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08GHC4-165	17.98	83.81	215.09	43.82	64.00	15.88	15.20
08GHC4-166	28.46	69.10	176.18	32.78	59.78	13.23	13.54
08GHC4-167	19.36	72.64	259.01	41.77	62.05	15.97	16.27
08GHC4-169	26.86	73.48	235.95	53.88	65.75	18.35	13.57
08GHC4-170	19.93	81.35	216.53	52.19	66.63	17.38	19.54
08GHC4-171	28.42	69.73	197.20	32.56	61.37	12.44	15.35
08GHC4-172	17.37	75.77	202.56	35.54	60.67	13.88	15.66
08GHC4-173	24.75	77.00	246.97	47.04	68.66	14.68	11.90
08GHC4-174	28.25	82.79	249.55	64.57	63.70	23.32	15.95
08GHC4-176	29.30	75.66	237.95	49.39	63.62	17.84	18.00
08GHC4-180	28.14	85.60	213.27	41.45	67.23	13.61	16.57
08GHC4-181	23.35	62.65	240.64	48.00	64.26	16.77	19.60
08GHC4-183	21.63	69.90	212.69	50.73	61.48	19.26	19.10
08GHC4-184	25.65	74.57	181.99	42.92	63.22	15.19	14.08
08GHC4-185	23.74	72.22	243.38	41.10	62.51	15.52	14.53
08GHC4-186	32.74	74.51	245.17	44.48	63.16	16.42	17.17
08GHC4-187	25.31	74.12	256.95	56.14	64.00	20.11	16.62
08GHC4-188	29.98	69.68	220.75	43.85	66.54	14.61	13.54
08GHC4-190	24.89	69.72	156.19	25.56	59.81	10.04	14.99
08GHC4-191	29.01	74.20	233.42	51.33	69.44	15.41	15.04
08GHC4-192	23.57	81.72	192.80	30.63	66.41	10.03	11.50
08GHC4-193	26.77	73.82	174.73	46.83	63.29	17.04	11.68
08GHC4-194(1)	23.41	71.63	222.70	36.11	61.91	14.00	16.67
08GHC4-194(2)	23.02	78.23	210.58	43.35	65.89	14.94	11.61
08GHC4-195	33.65	73.30	275.28	49.84	64.40	17.70	11.68
08GHC4-197	28.82	68.79	217.97	46.99	65.84	16.02	17.12
08GHC4-198	16.79	79.14	186.16	39.56	67.58	12.77	16.11
08GHC4-200	12.15	74.40	245.91	50.20	64.65	17.85	16.05
08GHC4-201	30.45	78.56	222.77	51.43	65.33	17.74	17.54
08GHC4-202	15.67	81.24	156.78	40.71	65.48	14.22	12.65
08GHC4-203	19.82	64.23	193.63	30.19	57.74	12.80	17.08
08GHC4-204	24.24	71.93	181.40	35.80	58.28	14.82	14.40
09178(1)(P1)	21.83	78.45	246.66	48.05	64.85	17.00	14.82
N99(P2)	24.44	70.73	213.87	36.50	62.64	13.73	14.44
Mean	**23.90**	**73.98**	**222.34**	**43.36**	**63.91**	**15.46**	**15.03**
SE	**2.29**	**1.57**	**13.66**	**5.15**	**1.92**	**1.84**	**1.12**
Line Mean	**23.91**	**73.97**	**222.25**	**43.37**	**63.91**	**15.46**	**15.04**

Appendix 11. Least square means for plant stand, anthesis date, plant height, wet weight, moisture content, total biomass, and brix at Mead, Nebraska for the 2008 season.

Mead 2008							
Line	**PS**	**AD**	**PH**	**WWT**	**MC**	**BY**	**Brix**
08GHC4-1	21.14	99.95	209.31	46.16	62.95	17.24	18.81
08GHC4-2	24.78	93.30	251.79	49.03	63.61	17.85	18.17
08GHC4-5	21.93	69.68	220.25	30.08	60.97	11.75	19.27
08GHC4-6	25.29	71.52	220.07	30.86	59.20	12.49	18.78
08GHC4-7	23.64	69.98	239.04	35.24	61.16	13.65	19.38
08GHC4-8	21.33	71.90	224.75	25.81	56.68	10.69	19.06
08GHC4-9	24.27	72.12	235.93	29.66	61.45	11.39	19.34
08GHC4-10	25.01	67.77	201.52	24.57	61.36	9.47	16.24
08GHC4-11	25.40	72.00	270.33	42.08	60.87	16.40	13.11
08GHC4-12	19.35	70.63	213.96	23.01	59.02	9.55	17.57
08GHC4-13	23.94	73.65	233.75	29.08	53.06	13.47	16.91
08GHC4-14	21.31	67.14	229.23	28.12	58.57	11.97	19.69
08GHC4-15	23.19	71.35	241.52	33.20	59.55	13.24	20.28
08GHC4-16	21.80	71.71	239.47	29.96	60.33	11.99	16.90
08GHC4-17	21.39	75.22	195.79	27.83	61.21	10.74	16.99
08GHC4-19	17.33	69.49	177.41	14.41	48.70	6.75	14.60
08GHC4-20	21.58	72.49	179.75	26.60	54.26	12.33	17.19
08GHC4-21	24.34	74.20	173.74	21.81	60.42	8.74	18.85
08GHC4-22	24.22	76.08	242.08	41.04	59.98	16.41	15.61
08GHC4-23	25.59	75.29	237.19	32.90	62.67	12.37	16.21
08GHC4-24	22.47	77.34	178.34	22.59	62.83	8.27	17.06
08GHC4-26	25.71	77.94	248.77	29.59	62.77	10.95	16.05
08GHC4-27	26.42	75.45	246.56	36.92	62.19	13.94	16.68
08GHC4-28	24.29	71.55	172.80	28.15	59.97	11.13	17.29
08GHC4-30	23.68	72.46	226.51	22.80	55.55	9.98	15.99
08GHC4-31	24.58	69.31	176.98	21.20	58.03	9.17	16.94
08GHC4-32	15.38	74.05	261.88	41.68	64.82	14.49	17.04
08GHC4-33	22.91	73.73	232.63	23.56	58.06	10.26	17.51
08GHC4-34	22.13	75.91	249.10	27.64	60.69	10.54	15.57
08GHC4-35	25.56	69.09	208.07	26.44	56.62	11.67	16.30
08GHC4-36	18.60	72.86	209.11	39.17	64.26	13.89	14.72
08GHC4-37	25.24	71.63	233.89	21.06	56.62	9.30	14.37
08GHC4-38	21.82	72.57	243.05	41.53	63.23	15.25	18.14
08GHC4-39	23.43	74.47	260.46	38.12	62.13	14.47	18.13
08GHC4-40	20.37	73.10	215.49	34.31	59.29	13.97	18.33
08GHC4-41	18.77	74.82	224.83	22.14	60.00	8.75	17.29
08GHC4-42	25.09	76.51	240.93	28.19	59.85	11.55	14.72
08GHC4-44	18.40	75.58	234.08	28.58	63.24	10.50	18.01
08GHC4-45	24.88	75.31	206.77	31.33	61.25	12.23	18.05
08GHC4-46	23.19	73.89	189.87	29.76	63.10	10.89	21.15
08GHC4-47	22.49	75.51	178.02	28.63	58.43	11.69	15.58
08GHC4-48	24.14	72.01	223.88	35.21	56.12	15.49	17.33
08GHC4-49	20.37	73.34	169.67	28.92	61.85	10.91	12.51

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08GHC4-50	21.09	77.23	214.00	31.61	60.36	12.52	17.88
08GHC4-51	13.44	74.62	174.78	32.39	55.63	14.23	15.79
08GHC4-52	21.02	77.67	181.84	28.77	58.37	11.88	17.21
08GHC4-53	21.56	75.64	255.17	25.28	57.37	10.95	15.66
08GHC4-54	20.85	79.73	247.11	31.38	62.71	11.77	16.58
08GHC4-55	22.25	72.25	176.04	24.92	56.76	10.97	16.95
08GHC4-56	20.40	72.58	250.14	30.47	64.58	10.71	13.36
08GHC4-57	22.89	75.69	243.05	35.57	59.62	14.43	15.92
08GHC4-59	25.94	74.09	224.26	17.26	49.70	8.27	14.79
08GHC4-62	23.87	71.88	203.66	30.12	67.84	9.63	19.49
08GHC4-63	25.42	78.36	257.04	29.79	63.04	10.97	15.91
08GHC4-64	24.11	71.74	244.99	23.19	62.35	8.89	18.45
08GHC4-65	20.37	74.57	218.47	37.57	59.22	15.34	16.65
08GHC4-66	24.43	75.95	244.95	37.33	60.52	14.77	16.29
08GHC4-67	23.67	79.31	227.92	35.50	64.27	12.62	18.47
08GHC4-68	17.62	74.99	264.06	28.53	61.12	11.13	16.86
08GHC4-69	16.41	74.00	246.44	32.28	59.01	13.11	16.72
08GHC4-70	22.81	73.47	199.19	23.72	58.28	9.75	16.30
08GHC4-71	23.61	74.91	245.61	37.33	57.74	15.92	17.97
08GHC4-72	20.05	74.84	241.59	35.38	65.79	11.99	12.51
08GHC4-73	24.14	72.39	235.74	31.42	62.67	11.75	14.39
08GHC4-76	21.62	70.15	199.01	24.21	58.14	10.20	17.09
08GHC4-77	21.34	74.00	251.92	37.07	61.21	14.11	17.89
08GHC4-78	23.18	73.43	218.16	31.07	59.83	12.45	17.98
08GHC4-79	20.23	72.87	226.91	32.71	62.35	12.18	16.36
08GHC4-80	23.10	69.94	169.51	20.08	56.05	8.84	17.16
08GHC4-81	26.42	68.40	184.31	22.47	58.55	9.23	18.09
08GHC4-82	22.66	72.44	240.25	35.05	61.55	13.38	19.31
08GHC4-83	22.34	72.99	239.25	30.52	62.74	11.36	15.53
08GHC4-84	22.30	75.04	236.79	28.42	64.61	10.00	16.32
08GHC4-86	25.16	70.96	214.39	26.78	57.93	11.18	18.22
08GHC4-87	23.42	77.86	242.96	28.48	60.36	11.23	19.78
08GHC4-88	22.29	76.15	206.62	26.18	60.08	10.10	16.46
08GHC4-89	24.39	69.50	195.03	24.21	60.00	9.46	15.58
08GHC4-90	23.87	73.55	185.74	30.13	59.91	12.10	15.36
08GHC4-91	25.37	84.55	196.52	27.87	61.50	10.61	17.31
08GHC4-92	20.63	73.94	214.87	35.22	61.07	13.78	18.65
08GHC4-93	24.34	72.39	208.06	24.59	53.06	11.45	18.00
08GHC4-95	19.72	75.17	197.95	33.45	63.25	12.23	16.13
08GHC4-96	23.34	78.52	242.60	42.27	61.69	16.19	17.06
08GHC4-97	20.72	72.44	244.35	30.98	61.04	11.95	13.75
08GHC4-99	21.93	79.03	228.10	22.37	61.89	8.29	18.21
08GHC4-100	23.15	79.44	264.94	44.48	63.30	16.27	17.08
08GHC4-102	17.60	79.37	225.15	35.80	61.21	13.69	19.30
08GHC4-103	25.06	74.76	250.61	28.86	57.16	12.27	15.54

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08GHC4-104	25.02	72.98	238.40	27.92	59.50	11.21	15.57
08GHC4-105	23.61	72.54	245.19	30.91	57.46	13.37	16.00
08GHC4-106	20.36	77.19	239.76	28.62	59.55	11.42	18.31
08GHC4-108	22.25	72.03	258.57	26.97	57.42	11.52	18.25
08GHC4-109	25.40	71.45	189.36	18.36	60.10	7.35	11.34
08GHC4-110	23.15	74.90	265.60	36.36	60.55	14.18	16.87
08GHC4-112	22.81	75.98	223.36	28.07	64.03	9.96	19.09
08GHC4-113	23.11	72.51	223.70	26.17	59.35	10.67	14.39
08GHC4-114	23.30	70.55	216.21	23.54	52.18	10.75	9.74
08GHC4-116	23.06	76.64	274.23	35.13	60.10	14.08	17.35
08GHC4-117	21.77	71.84	236.34	28.91	60.08	11.51	16.91
08GHC4-118	22.57	74.84	241.68	42.85	63.42	15.70	17.70
08GHC4-125	20.35	75.47	212.98	33.71	58.67	14.04	18.80
08GHC4-126	23.93	72.70	226.76	24.72	62.07	9.41	14.71
08GHC4-127	26.19	69.38	199.03	26.47	55.51	11.51	19.74
08GHC4-128	22.63	70.51	258.39	25.11	60.92	9.76	17.21
08GHC4-129	25.67	68.11	217.70	27.09	65.02	9.36	13.60
08GHC4-130	20.56	73.06	231.33	37.40	60.60	14.71	17.93
08GHC4-131	21.49	74.83	231.65	38.56	67.38	12.67	11.33
08GHC4-132	23.55	70.90	238.42	31.49	61.19	12.12	18.15
08GHC4-133	23.83	68.02	219.92	26.76	59.57	10.67	18.62
08GHC4-134	22.31	70.51	220.86	35.59	60.56	13.76	19.09
08GHC4-135	15.35	72.11	255.29	37.99	64.10	13.55	16.07
08GHC4-136	23.55	70.81	255.65	31.44	63.80	11.28	18.89
08GHC4-137	27.08	68.08	186.68	21.25	59.58	8.58	20.48
08GHC4-140	24.31	70.45	237.46	34.09	60.31	13.32	15.29
08GHC4-141	22.85	74.13	236.02	27.14	61.97	10.34	19.54
08GHC4-142	21.92	71.25	238.89	38.12	60.81	15.05	19.61
08GHC4-143	25.91	79.00	263.50	48.78	60.66	19.09	16.66
08GHC4-145	19.36	78.45	185.61	30.75	63.61	11.28	13.75
08GHC4-147	24.47	70.88	243.69	29.28	64.59	10.40	16.57
08GHC4-148	21.39	71.48	277.17	28.49	63.16	10.49	15.02
08GHC4-149	22.85	75.92	185.28	23.03	60.25	8.89	16.35
08GHC4-150	26.27	76.59	192.20	28.12	63.55	10.20	17.99
08GHC4-151	24.29	76.04	187.49	30.26	56.76	12.72	17.63
08GHC4-152	25.08	73.56	216.25	31.57	62.33	11.87	16.36
08GHC4-153	22.57	74.35	246.74	32.52	62.37	12.33	15.35
08GHC4-154	19.38	71.55	282.82	46.07	61.68	17.65	16.17
08GHC4-157	24.69	72.00	215.32	30.59	61.33	11.55	17.88
08GHC4-159	25.87	72.87	247.18	31.54	57.70	13.11	17.42
08GHC4-160	21.66	69.69	204.69	19.51	62.96	7.24	14.29
08GHC4-161	26.14	76.52	258.94	29.13	60.21	11.61	16.66
08GHC4-163	23.92	73.64	263.65	38.82	60.25	15.52	15.99
08GHC4-164	19.38	86.78	186.04	42.99	63.52	15.72	16.08
08GHC4-165	24.12	72.46	206.54	27.31	64.30	9.74	17.26

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08GHC4-166	22.37	70.06	192.58	20.22	53.97	9.09	15.15
08GHC4-167	22.79	80.00	277.96	43.97	62.21	16.52	17.96
08GHC4-169	26.51	72.05	238.16	29.66	56.29	12.88	17.22
08GHC4-170	23.10	74.92	255.30	32.72	63.38	11.87	15.73
08GHC4-171	18.58	75.51	249.62	41.60	64.85	14.53	14.75
08GHC4-172	20.53	73.19	243.92	42.14	64.27	15.18	18.03
08GHC4-173	14.77	72.10	263.28	32.84	61.38	12.67	15.17
08GHC4-174	20.47	75.09	208.62	26.90	61.40	10.13	13.65
08GHC4-176	22.91	71.03	228.95	24.98	63.18	9.19	15.38
08GHC4-180	24.30	72.52	271.29	41.86	63.55	15.20	18.74
08GHC4-181	24.48	69.62	222.14	23.08	58.30	9.54	16.01
08GHC4-183	20.86	68.92	226.31	21.25	63.58	7.71	13.90
08GHC4-184	16.95	72.88	265.58	37.46	63.47	13.60	17.39
08GHC4-185	24.50	66.83	175.27	14.49	58.43	6.06	19.33
08GHC4-186	21.69	68.41	192.38	19.29	61.30	7.32	19.93
08GHC4-187	23.03	69.16	157.78	20.06	54.53	9.27	17.25
08GHC4-188	23.69	72.99	259.26	39.87	63.28	14.50	16.64
08GHC4-190	25.13	75.49	202.65	27.68	57.69	11.66	12.68
08GHC4-191	24.06	68.00	183.85	16.26	56.37	6.81	18.48
08GHC4-192	24.39	74.23	254.33	37.17	63.96	13.33	15.79
08GHC4-193	24.53	68.98	255.03	29.89	63.30	10.96	17.37
08GHC4-194(1)	18.22	72.92	264.62	27.73	60.26	11.06	17.74
08GHC4-194(2)	23.67	71.90	253.90	27.06	56.62	11.71	17.16
08GHC4-195	25.09	70.56	232.19	29.32	60.78	11.63	18.16
08GHC4-197	25.93	73.03	232.10	14.74	54.86	6.16	9.57
08GHC4-198	22.15	69.34	240.49	33.75	63.62	12.26	15.15
08GHC4-200	25.35	74.95	318.79	48.55	60.55	19.27	18.85
08GHC4-201	.	.	.	.	.	.	.
08GHC4-202	20.76	72.39	238.47	29.51	59.70	11.93	15.63
08GHC4-203	22.81	78.93	281.97	36.08	63.52	13.13	17.31
08GHC4-204	21.08	69.97	163.01	19.11	55.38	8.76	16.64
09178(1)(P1)	19.76	84.27	239.54	45.08	63.95	16.27	17.40
N99(P2)	25.65	71.56	239.05	29.89	62.01	11.26	17.82
Mean	**22.64**	**73.72**	**227.03**	**30.52**	**60.47**	**11.91**	**16.79**
SE	**2.29**	**1.57**	**13.66**	**5.15**	**1.92**	**1.84**	**1.12**
Line Mean	**22.64**	**73.67**	**226.88**	**30.43**	**60.44**	**11.88**	**16.78**

Appendix 12. Least square means for plant stand, anthesis date, plant height, wet weight, moisture content, total biomass, and brix at Mead, Nebraska for the 2009 season.

Mead 2009							
Line	**PS**	**AD**	**PH**	**WWT**	**MC**	**BY**	**Brix**
08GHC4-1	25.29	64.40	288.87	41.85	67.44	13.73	14.25
08GHC4-2	29.67	73.24	299.54	44.74	64.95	15.58	16.60
08GHC4-5	25.35	75.12	274.71	55.42	73.94	14.06	15.52
08GHC4-6	22.81	71.81	293.65	51.33	67.43	16.70	17.21
08GHC4-7	21.10	72.43	276.51	29.61	67.87	9.35	11.49
08GHC4-8	23.24	64.28	166.25	29.11	64.55	9.89	9.05
08GHC4-9	25.45	73.84	289.61	55.59	63.36	19.94	16.63
08GHC4-10	19.17	68.65	277.18	41.12	63.53	15.01	13.78
08GHC4-11	23.07	71.03	308.12	39.81	66.72	13.15	12.79
08GHC4-12	18.34	69.53	273.11	48.92	65.37	16.45	16.34
08GHC4-13	30.57	68.72	286.73	45.19	63.77	16.41	16.69
08GHC4-14	22.45	72.53	206.62	52.20	70.26	15.56	11.55
08GHC4-15	27.96	84.32	245.48	34.81	65.82	11.95	14.87
08GHC4-16	25.08	73.50	278.14	48.81	65.78	16.63	15.47
08GHC4-17	24.96	72.97	284.71	50.42	62.89	18.55	14.22
08GHC4-19	27.00	74.79	255.20	44.63	69.85	13.53	12.59
08GHC4-20	25.79	73.87	307.38	67.41	60.30	27.03	23.70
08GHC4-21	26.70	74.43	250.81	51.21	63.44	18.70	16.45
08GHC4-22	30.40	78.00	234.16	25.29	66.94	8.45	6.62
08GHC4-23	20.88	70.57	245.07	43.27	71.36	12.54	17.03
08GHC4-24	29.95	72.51	239.84	30.08	63.65	10.94	15.63
08GHC4-26	25.60	73.20	287.39	48.65	65.04	16.84	16.18
08GHC4-27	25.00	79.60	210.68	20.31	67.90	6.28	11.19
08GHC4-28	25.88	71.14	283.23	54.46	68.30	16.97	15.41
08GHC4-30	22.95	72.51	282.08	57.10	67.38	18.55	14.81
08GHC4-31	25.89	66.69	268.27	39.84	66.00	13.77	15.34
08GHC4-32	22.16	70.08	273.47	51.75	64.46	18.13	14.99
08GHC4-33	26.67	80.59	225.53	53.45	65.95	18.29	14.29
08GHC4-34	27.81	73.63	259.78	36.14	66.01	11.51	15.62
08GHC4-35	28.50	68.46	253.48	48.71	68.39	15.43	15.73
08GHC4-36	24.39	71.06	276.27	40.08	66.87	13.36	14.18
08GHC4-37	20.01	77.57	250.61	31.74	62.70	12.02	15.41
08GHC4-38	27.39	87.97	254.05	54.49	64.44	19.23	12.53
08GHC4-39	25.24	70.13	244.95	44.01	67.13	14.37	15.41
08GHC4-40	22.39	75.61	247.75	46.61	64.54	16.38	17.74
08GHC4-41	22.82	67.13	265.24	41.39	67.64	13.53	13.79
08GHC4-42	22.04	72.07	266.26	57.47	64.67	20.43	16.52
08GHC4-44	28.53	68.12	175.85	25.76	63.75	9.36	14.40
08GHC4-45	26.20	78.88	261.20	41.85	62.84	15.46	15.26
08GHC4-46	23.38	71.19	269.13	33.05	62.53	12.37	15.66
08GHC4-47	25.94	74.26	275.37	65.42	67.74	20.84	16.17
08GHC4-48	22.37	68.46	262.61	49.18	68.38	15.57	15.95

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08GHC4-49	24.88	74.50	242.03	32.29	61.73	12.26	15.98
08GHC4-50	24.92	65.55	246.95	46.38	65.21	16.19	16.23
08GHC4-51	25.42	67.16	245.51	40.43	66.09	13.67	11.19
08GHC4-52	28.40	61.16	277.38	37.36	66.85	12.37	16.35
08GHC4-53	25.13	73.56	216.05	33.14	64.86	11.68	15.50
08GHC4-54	25.03	76.72	249.08	30.82	65.11	10.77	13.26
08GHC4-55	27.19	84.57	279.28	49.62	64.41	17.72	15.16
08GHC4-56	24.20	74.63	267.52	53.76	65.19	18.64	15.89
08GHC4-57	30.38	79.48	244.40	57.55	67.04	18.83	14.51
08GHC4-59	21.28	67.72	261.79	43.09	66.14	14.35	15.79
08GHC4-62	29.02	71.22	227.23	50.47	64.83	17.84	16.52
08GHC4-63	21.97	68.99	209.33	31.27	67.74	9.99	15.56
08GHC4-64	22.84	68.39	270.41	38.81	58.35	15.63	13.39
08GHC4-65	28.07	73.71	248.69	50.32	63.97	17.95	16.52
08GHC4-66	21.35	67.64	274.03	45.00	67.50	14.56	15.93
08GHC4-67	27.98	61.12	263.08	37.20	68.64	11.66	14.69
08GHC4-68	30.03	73.26	266.87	60.53	64.66	21.32	16.44
08GHC4-69	25.17	67.90	256.93	44.58	67.95	14.43	16.29
08GHC4-70	22.81	73.61	282.48	45.32	66.01	15.51	15.37
08GHC4-71	24.53	70.04	243.39	34.76	65.78	11.59	17.67
08GHC4-72	31.78	74.21	278.01	56.47	65.59	19.54	15.49
08GHC4-73	24.36	70.89	281.54	38.53	61.35	14.65	13.81
08GHC4-76	24.33	72.66	259.04	49.22	64.63	17.48	17.00
08GHC4-77	27.76	73.26	203.12	31.60	62.84	11.49	15.10
08GHC4-78	22.00	75.12	269.22	58.71	62.42	22.07	13.31
08GHC4-79	24.51	73.10	185.09	34.64	69.87	10.56	16.46
08GHC4-80	31.33	74.56	213.67	44.05	68.36	14.02	15.74
08GHC4-81	25.32	76.75	208.77	31.93	65.83	11.04	13.71
08GHC4-82	22.64	81.61	259.12	38.26	65.76	13.17	16.28
08GHC4-83	26.56	75.44	289.03	47.81	66.66	15.90	15.74
08GHC4-84	16.01	80.05	237.85	31.97	65.38	11.15	13.84
08GHC4-86	27.34	73.53	207.19	37.80	67.34	12.37	16.02
08GHC4-87	25.41	83.97	200.68	39.77	66.71	13.23	14.68
08GHC4-88	25.98	71.95	256.38	50.57	60.97	20.16	17.02
08GHC4-89	18.32	79.68	268.15	69.92	70.46	20.70	14.70
08GHC4-90	26.12	69.79	203.26	23.59	60.69	9.45	14.78
08GHC4-91	19.37	71.33	262.84	59.00	60.63	23.04	13.99
08GHC4-92	22.41	69.21	258.36	48.09	65.23	16.61	15.55
08GHC4-93	27.08	66.83	280.51	60.27	63.79	21.94	14.30
08GHC4-95	27.60	62.44	203.82	36.09	75.55	9.14	16.29
08GHC4-96	25.94	78.07	283.54	44.27	68.26	14.01	9.34
08GHC4-97	27.64	74.59	196.81	37.87	68.55	11.88	16.01
08GHC4-99	28.16	74.54	235.71	32.75	69.31	9.76	13.84
08GHC4-100	24.21	73.21	223.93	32.46	66.62	10.69	11.60
08GHC4-102	21.91	78.24	262.78	39.15	66.93	12.76	16.80

Appendix 12. Cont'd

08GHC4-103	21.20	89.53	249.17	34.72	62.96	13.00	15.29
08GHC4-104	25.70	70.97	258.50	48.90	69.24	14.96	17.23
08GHC4-105	23.97	73.29	278.00	47.88	64.57	16.97	17.39
08GHC4-106	28.27	61.87	174.43	38.40	67.60	12.08	13.85
08GHC4-108	23.16	78.38	283.66	53.51	63.14	19.70	13.80
08GHC4-109	25.05	63.74	229.61	36.45	65.85	12.20	12.14
08GHC4-110	29.64	81.19	278.32	45.36	66.85	15.05	14.78
08GHC4-112	29.74	77.64	281.97	56.95	62.59	21.13	17.54
08GHC4-113	21.85	75.23	268.52	47.58	64.67	16.74	16.67
08GHC4-114	23.02	71.74	275.04	46.01	58.73	18.86	17.64
08GHC4-116	24.77	75.35	294.62	69.18	63.81	25.36	15.55
08GHC4-117	22.68	73.11	279.57	46.93	64.06	16.77	14.32
08GHC4-118	27.52	70.46	246.61	24.25	64.99	8.47	14.59
08GHC4-125	27.39	81.01	184.42	35.92	67.48	11.59	13.98
08GHC4-126	27.28	78.19	314.24	57.19	63.97	20.49	16.76
08GHC4-127	25.28	71.08	302.47	63.40	66.26	21.32	16.95
08GHC4-128	27.50	74.08	263.33	38.15	64.02	13.84	11.93
08GHC4-129	23.36	71.93	271.56	45.08	63.99	16.04	15.35
08GHC4-130	29.53	72.42	259.00	51.79	64.13	18.54	16.50
08GHC4-131	26.82	74.14	173.54	25.00	71.57	6.65	15.01
08GHC4-132	26.21	81.15	259.28	50.57	65.85	17.28	14.34
08GHC4-133	25.61	76.85	287.28	64.68	65.19	22.83	16.77
08GHC4-134	31.48	77.30	301.50	64.66	64.57	22.71	17.49
08GHC4-135	28.62	64.05	225.78	33.45	67.73	10.78	12.42
08GHC4-136	24.27	66.52	243.48	14.45	64.21	5.39	12.26
08GHC4-137	23.13	72.50	263.31	43.97	62.48	16.46	13.92
08GHC4-140	21.80	68.56	269.22	47.61	62.45	17.61	15.66
08GHC4-141	26.69	69.49	271.07	60.33	67.27	19.76	17.65
08GHC4-142	21.19	82.18	282.68	41.55	41.47	20.83	18.21
08GHC4-143	23.63	69.15	256.55	44.60	67.08	14.48	16.30
08GHC4-145	27.71	72.53	246.80	60.93	63.89	22.21	15.62
08GHC4-147	25.13	69.29	253.81	39.49	65.08	13.61	12.08
08GHC4-148	28.48	82.76	301.79	82.25	65.23	27.78	16.78
08GHC4-149	21.85	79.60	206.41	21.67	60.50	8.62	7.35
08GHC4-150	26.68	68.93	247.67	53.27	63.33	19.46	16.85
08GHC4-151	25.58	67.55	240.21	36.70	67.32	11.98	16.31
08GHC4-152	23.56	69.00	270.78	47.94	67.31	15.65	14.81
08GHC4-153	24.84	78.51	286.91	55.46	67.34	18.07	14.37
08GHC4-154	20.61	79.22	259.15	55.15	70.63	16.14	12.42
08GHC4-157	25.00	67.12	315.74	48.50	66.76	16.12	17.51
08GHC4-159	21.37	80.46	237.27	42.34	61.34	15.66	16.89
08GHC4-160	20.25	68.75	178.62	17.04	61.20	6.32	14.92
08GHC4-161	24.30	77.51	230.48	41.28	67.64	13.40	17.44
08GHC4-163	29.18	64.37	249.45	23.76	65.72	8.39	13.21

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08GHC4-164	24.21	76.19	250.09	39.12	67.03	13.13	16.46
08GHC4-165	30.19	69.54	248.48	52.40	68.93	16.45	17.30
08GHC4-166	18.79	77.45	224.55	43.80	67.01	14.41	15.51
08GHC4-167	21.24	73.43	254.89	45.35	67.56	14.56	15.62
08GHC4-169	25.21	79.54	279.61	52.86	60.58	21.06	15.02
08GHC4-170	27.02	68.76	259.44	32.29	64.07	11.71	14.13
08GHC4-171	23.18	79.07	289.88	53.93	64.26	19.12	18.85
08GHC4-172	24.52	72.74	243.18	42.37	64.30	15.29	16.42
08GHC4-173	23.16	74.25	249.15	53.15	64.97	18.04	17.78
08GHC4-174	23.23	79.53	255.66	45.70	66.09	15.36	11.09
08GHC4-176	23.19	74.17	219.38	25.27	66.38	8.79	13.18
08GHC4-180	23.32	67.68	253.67	44.19	60.29	17.18	13.49
08GHC4-181	27.74	69.63	206.66	39.32	69.35	12.09	17.50
08GHC4-183	28.08	69.79	200.43	27.59	64.59	9.71	10.70
08GHC4-184	24.40	73.80	278.08	43.31	63.38	15.75	15.90
08GHC4-185	26.71	72.66	274.61	38.22	70.66	11.17	11.54
08GHC4-186	25.49	78.46	235.64	30.28	63.53	10.60	16.66
08GHC4-187	22.30	70.53	204.74	38.67	68.13	12.41	14.35
08GHC4-188	28.49	72.95	253.05	49.04	69.62	14.98	11.84
08GHC4-190	24.91	62.86	175.82	19.71	64.90	6.64	12.60
08GHC4-191	26.93	77.60	279.02	59.07	66.87	19.63	17.05
08GHC4-192	25.60	72.05	293.35	52.11	66.93	17.17	15.63
08GHC4-193	30.16	71.61	288.44	49.83	65.25	17.15	16.98
08GHC4-194(1)	25.59	69.69	229.47	46.56	67.51	15.04	17.01
08GHC4-194(2)	22.79	69.37	207.89	26.71	69.39	8.13	9.47
08GHC4-195	27.64	67.15	231.18	33.80	67.87	10.83	16.24
08GHC4-197	22.72	69.85	214.44	15.79	63.13	5.64	13.58
08GHC4-198	20.27	73.58	249.31	25.51	64.35	8.90	14.29
08GHC4-200	21.63	78.65	274.70	60.33	64.17	21.43	17.29
08GHC4-201	30.55	67.04	289.78	47.03	67.29	15.25	14.37
08GHC4-202	24.93	75.68	290.23	46.91	62.06	17.84	15.34
08GHC4-203	23.99	69.43	289.57	41.29	67.96	13.35	14.77
08GHC4-204	24.24	78.23	264.86	34.72	65.36	11.95	15.17
09178(1)(P1)	23.98	82.05	287.51	49.36	64.06	17.92	14.97
N99(P2)	23.53	79.84	271.22	49.18	68.62	15.18	13.99
Mean	**25.07**	**73.12**	**254.49**	**43.79**	**65.53**	**15.01**	**15.03**
SE	**2.29**	**1.57**	**13.66**	**5.15**	**1.92**	**1.84**	**1.12**
Line Mean	**25.09**	**73.03**	**254.19**	**43.72**	**65.52**	**14.99**	**15.04**